FINAL REGISTRATION REPORT

Part B

Section 5

Analytical Methods

Detailed summary of the risk assessment

Product code: A-200SL-OR3-C

Product name(s): **LEPTOSAR 200 SL**

Chemical active substance:

Acetamiprid, 200 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: CIECH Sarzyna S.A.

Submission date: 02/2021

MS Finalisation date: 01/07/2022

Version history

When	What
February 2021	First submission of product authorization.
May 2021	Dossier sent for evaluation
October 2021	Correction of first submission for product authorization in Poland.
December 2021	zRMS finalised evaluation
July 2022	Final version prepared by zRMS after Commenting period

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Evaluator comments:
The text highlighted in grey was provided by the evaluator.

5 Analytical methods

5.1 Conclusion and summary of assessment

Sufficiently sensitive and selective analytical methods are available for the active substance in the plant protection product.
Noticed data gaps are:

- ~~data gap 1~~
- ~~data gap 2~~
- ~~data gap 3~~

Sufficiently sensitive and selective analytical methods are available for all analytes included in the residue definitions.
The applicant relevant text was not rewritten. The zRMS comments are on grey background.
It was concluded that adequate analytical methods are available to monitor acetamiprid in food of plant, animal origin, in soil, water and air. All these analytical methods are active substance data and were provided in the EU review of acetamiprid. However, additional data on all these methods and in other matrixes in support of ecotoxicological studies have been provided. The validated methods for determination of the relevant acetamiprid metabolites were also provided. All data were considered adequate.
In addition, EFSA (EJ 2021;19(9):6830) notes that the extraction efficiency for the analytical methods applied for enforcement and used for the residue trials is not sufficiently proven for all commodities groups according to the requirements (SANTE 2017/10632. Further investigation on this matter would in principle be required.
In the context of the applicant's authorisation request noticed data gaps are: none
In the context of the residue analytical methods required the product can be authorised.

Commodity/crop	Supported/ Not supported
Winter oilseed rape	supported
Maize	supported
Soft wheat, Hard wheat, Spelt wheat, Rye	supported
Spring oilseed rape, white mustard, black mustard, Chinese mustard, turnip rape	supported
Flax	supported
Common hemp	supported
Soybean	supported
Opium poppy	supported

5.2 Methods used for the generation of pre-authorization data (KCP 5.1)

5.2.1 Analysis of the plant protection product (KCP 5.1.1)

5.2.1.1 Determination of active substance and/or variant in the plant protection product (KCP 5.1.1)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in plant protection product is provided as follows:

Comments of zRMS:	The method is accepted and may be applied for active substance analysis in the PPP.

Reference:	KCP 5.1.1
Report	A-200SL-OR3-C, Part I: Determination of physicochemical properties of the initial preparation after accelerated storage, E. Arévalo,2019, BF-24/19
Guideline(s):	Yes (SANCO/3030/99 rev. 5)
Deviations:	No
GLP:	No (GLP was not compulsory for analytical methods)
Acceptability:	Yes

Materials and methods

The determination of the active ingredient content – acetamiprid – in A-200SL-OR3-C preparation was carried out using reversed phase high performance liquid chromatography (RP-HPLC) with UV-DAD detection at wavelength 247 nm and external standard. The determination was carried out under following chromatographic conditions:

- Oven temperature 35 ºC
- Mobile phase flow v = 1.0 mL/min
- Wavelength λ = 247 nm
- Injection volume 2 µL
- Mobile phase composition: acetonitrile + 2.0% CH_3COOH (40 + 60, v/v)
- Under the above conditions retention time of acetamiprid is about 3.0 min. Total analysis time is 7.5 minutes.

Validation - Results and discussions

Table 5.2-1: Methods suitable for the determination of acetamiprid in plant protection product LEPTOSAR 200 SL

	Acetamiprid
Author(s), year	E. Arévalo, 2019
Principle of method	The determination of the active ingredient content – acetamiprid – in A-200 SL-OR3-C preparation was carried out using reversed phase high performance liquid chromatography (RP-HPLC) with UV-DAD detection at wavelength 247 nm and external standard.
Linearity (linear between mg/L / % range of the declared content)	The linearity of the analytical method was assessed using ten standard solutions in the concentration range from 0.2101 mg/mL to 0.4894 Correlation coefficient: $R^2 = 0.9993$. acceptance criterion ≥0.99

	Acetamiprid
(correlation coefficient, expressed as r)	Linear equation: y = 9916451.3992x + 54568.8329
Precision – Repeatability Mean n = 6 (%RSD)	%RSD = 0.31, acceptance criterion ≤ 1.76% Horrat = 0.18
Accuracy n = 6 (% Recovery)	99.1 % (mean) acceptance criterion 100% ± 3%
Interference/ Specificity	No interference
Comment	-

Conclusion

The method meets criteria required according to guideline SANCO/3030/99 which guarantee correctness of acetamiprid determination in the preparation LEPTOSAR 200 SL.

5.2.1.2 Description of analytical methods for the determination of relevant impurities (KCP 5.1.1)

LEPTOSAR 200 SL does not contain impurities which are of toxicological, ecotoxicological or environmental concern which could be arisen in the manufacturing process or as a result of degradation during storage of the product. It is not necessary to submit the analytical methods for determination of above mentioned impurities.

5.2.1.3 Description of analytical methods for the determination of formulants (KCP 5.1.1)

The other formulants and also components of other formulants of LEPTOSAR 200 SL are not of toxicological and/or ecotoxicological or environmental concern and therefore it is not necessary to submit the analytical methods for determination of other formulants or components of other formulants of above product.

5.2.1.4 Applicability of existing CIPAC methods (KCP 5.1.1)

There is no CIPAC method available for the determination of acetamiprid in SL formulation like LEPTOSAR 200 SL.

5.2.2 Methods for the determination of residues (KCP 5.1.2)

An overview on the acceptable methods and possible data gaps for analysis of residues of acetamiprid for the generation of pre-authorization data is given in the following table. For the detailed evaluation of new/ additional studies it is referred to Appendix 2.

Table 5.2-2: Validated methods for the generation of pre-authorization data

Component of residue definition: acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Maize, Oilseed rape,	Primary	0.01 mg/kg	HPLC-MS/MS	A. Markowicz. Determination of residues of acetamiprid in/on

Component of residue definition: acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Wheat (Residues)				maize under open field conditions following one application of A-200SL-OR3-CPD in Northern Europe in 2018 ZBBZ-2018/04/DPL/2, 2019
				A. Markowicz, Determination of residues of acetamiprid in/on winter/spring oilseed rape under open field conditions following one and two applications of A-200SL-OR3-CPD in Northern Europe in 2018 ZBBZ-2018/04/DPL/1, 2019
				A. Markowicz, Determination of residues of acetamiprid in/on oilseed rape under open field conditions following two applications of A-200SL-OR3-CPD in Northern Europe in 2019 428SRPL19R01, 2020
				A. Markowicz, Determination of residues of acetamiprid in/on winter wheat under open field conditions following one applications of A-200SL-OR3-CPD in Northern Europe in 2019 428SRPL19R02, 2020
Elendt M7 medium (Ecotoxicology)	Primary	0.0005 mg/kg	HPLC-DAD	E. Kulec-Płoszczyca , A-200SL-OR3-C, Daphnia magna, Acute immobilisation test, W/01/19, 2019
				P. Bąk, A-200SL-OR3-C, Chironomus sp., Acute immobilisation test, W/02/19, 2019
				K. Brzozowska-Wojoczek, A-200SL-OR3-C, Daphnia magna, Reproduction test, W/04/19, 2019
AAP medium (Ecotoxicology		0.0005 mg/kg	HPLC-DAD	E. Kulec-Płoszczyca, A-200SL-OR3-C, Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test, W/03/19, 2019
Water (Ecotoxicology)		0.1 mg/L	HPLC-DAD	M. Wołany, A-200SL-OR3-C, Terrestial Plant Test: Vegetative

Component of residue definition: acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
				Vigour Test, G/151/18, 2019
				M. Wołany, A-200SL-OR3-C, Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, G/152/18, 2019
Water, sucrose solution (Ecotoxicology)		0.1 mg/L (water) 0.2 mg/L (sucrose solution)	HPLC-DAD	M. Grzesica, A-200SL-OR3-C, Honeybees (*Apis mellifera* L.), Chronic Oral Toxicity Test, B/13/19, 2019
Water (Ecotoxicology)		0.06 mg /L water	HPLC-DAD	P.Holewik, A-200SL-OR3-C Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure, B-56-21, 2021

5.3 Methods for post-authorization control and monitoring purposes (KCP 5.2)

5.3.1 Analysis of the plant protection product (KCP 5.2)

Analytical methods for the determination of the active substance and relevant impurities in the plant protection product shall be submitted, unless the applicant shows that these methods already submitted in accordance with the requirements set out in point 5.2.1 can be applied.

5.3.2 Description of analytical methods for the determination of residues of acetamiprid (KCP 5.2)

5.3.2.1 Overview of residue definitions and levels for which compliance is required

Compared to the residue definition proposed in the Draft Assessment Report (incl. its addenda) the current legal residue definition is not identical.
Previously evaluated in original DAR definition in animal commodities: Acetamiprid and IM-2-1 metabolite
Existing definition in animal commodities: N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid
The current legal residue definition in plant commodities is identical.

Table 5.3-1: Relevant residue definitions for monitoring/enforcement and levels for which compliance is required

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, high water content	Acetamiprid	LOQ = 0.01 mg/kg	0.4 mg/kg in oilseed rape 0.01 mg/kg in maize
Plant, high acid content			
Plant, high protein/high starch content (dry commodities)			
Plant, high oil content			

Matrix	Residue definition	MRL / limit	Reference for MRL/level Remarks
Plant, difficult matrices (hops, spices, tea)			
Muscle	N-desmethyl-acetamiprid (IM-2-1), expressed as acetamiprid	LOQ = 0.01 mg/kg	0.02
Milk			0.2
Eggs			0.02
Fat			0.02
Liver, kidney			0.1
Soil (Ecotoxicology)	Acetamiprid	0.002 mg/kg	AOEL 0.025 mg/kg bw/d
Drinking water (Human toxicology)	Acetamiprid, IM-1-5	Acetamiprid 0.1 µg/L IM-1-5 0.05 µg/L	general limit for drinking water
Surface water (Ecotoxicology)	Acetamiprid	Acetamiprid 0.1 µg/L IM-1-5 0.05 µg/L	
Air	Acetamiprid	0.002 µg/m^3	AOEL 0.025 mg/kg bw/d
Tissue (meat or liver)	No residue definition provided	not required	not classified as T / T+
Body fluids		not required	not classified as T / T+

5.3.2.2 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in plant matrices is given in the following tables.

Table 5.3-2: Validated methods for food and feed of plant origin (required for all matrix types, "difficult" matrix only when indicated by intended GAP)

Component of residue definition: acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
Tobacco, cotton, pepper, pear, peach, orange and apple	Primary	LOQ = 0.01 mg/kg	GC-ECD	DAR 2001 Fuchsbichler G, 2000, HVA 21/00
Tomato, cucumber, plum, melon	Primary	LOQ = 0.01 mg/kg	GC-ECD	DAR 2001 Williams M., 1999, EC-97-388
Apples, tomatoes	Primary	LOQ = 0.01 mg/kg apples LOQ = 0.05 mg/kg tomatoes	GC-ECD	DAR 2001 Communal P.Y., 1997, RPA/NI-25/95101N
Cabbage, pears, raisins, cotton	Primary	LOQ = 0.01 mg/kg non citrus	GC-ECD	DAR 2001 Goller G., 1998, RPA/NI-

Component of residue definition: acetamiprid				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing / EU agreed**
seed, cotton gin, trash, broccoli, carrot root, orange whole fruit, orange oil		matrix LOQ = 0.05 mg/kg citrus		25/97062
High acid content (Oranges)	Primary	LOQ = 0.0104 mg/kg	UPLC-MS/MS	Longhi D., 2019, GLP-STUDY-18-000081
High oil content (Sunflower seeds)		LOQ = 0.0104 mg/kg		
Dry/high starch content (maize grain)		LOQ = 0.0104 mg/kg		
High acid content (Oranges)	ILV	LOQ = 0.01 mg/kg	HPLC/MS/MS	Ticco S. P., 2019, CH - 031/2019
High oil content (Sunflower seeds)		LOQ = 0.01 mg/kg		
Dry/high starch content (maize grain)		LOQ = 0.01 mg/kg		

Table 5.3-3: Statement on extraction efficiency

	Method for products of plant origin
Required, available from:	please refer to DAR section B.7.1
Not required, because:	-

5.3.2.3 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in animal matrices is given in the following tables.

Table 5.3-4: Validated methods for food and feed of animal origin (if appropriate)

Component of residue definition: acetamiprid (and metabolite IM-2-1[1])				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Milk, muscle, fat, kidney, liver	Primary	LOQ = 0.01 mg/kg for milk, muscle an fat LOQ = 0.05 mg/kg for liver and kidney	HPLC-UV	DAR 2001, xxx, 1997, 660216
Egg, muscle, fat, liver	Primary	LOQ = 0.01 mg/kg for	HPLC-UV	DAR 2001, xxx, 1997, 660227
Liver	Primary	LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)	LC-QQQ	xxx., 2017, DNA4036
Muscle		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Fat		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Milk		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Eggs		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Liver	ILV	LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)	LC-MS/MS	xxx, 2018, 133111101
Muscle		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Fat		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Milk		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		
Eggs		LOQ = 0.01 mg/kg (Acetamiprid and IM-2-1)		

[1] (E)-N-[(6-Chloro-3-pyridyl)methyl]-N'-cyanoacetamidine

Table 5.3-5: Statement on extraction efficiency

	Method for products of animal origin
Required, available from:	please refer to DAR section B.7.1
Not required, because:	-

5.3.2.4 Description of methods for the analysis of soil (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in soil is given in the following tables.

Table 5.3-6: Validated methods for soil (if appropriate)

Component of residue definition: acetamiprid (and metabolite IC-0, IM-1-4, IM-1-2, IM-1-5)			
Method type	**Method LOQ**	**Principle of method (*i.e.* GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ=0.01 mg/kg	LC/MS/MS	DAR 2001, Yang J., 1998, EC-97-411
Primary	LOQ=0.01 mg/kg	LC/MS/MS	Neal J.l, 2003, 02Y536660
Primary	LOQ=0.002 mg/kg (Acetamiprid, IM-1-4, IM-1-2)	LC-QQQ	Norris D., 2018, DNA4517
ILV	LOQ=0.002 mg/kg (Acetamiprid, IM-1-4, IM-1-2)	LC/MS/MS	Eichler M., Herrmannm S., 2018, 133113101

All analytical methods are active substance data and were evaluated during the EU review of acetamiprid. No additional studies have been performed.
These data have been provided and are considered to adequate.

5.3.2.5 Description of methods for the analysis of water (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in surface and drinking water is given in the following tables.

Table 5.3-7: Validated methods for water (if appropriate)

Component of residue definition: acetamiprid (and metabolite IM-1-5)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water River water, Ground water	Primary	LOQ = 0.1 µg/L	HPLC-UV	DAR 2001, Tokieda M., 1997, NCAS 97-007
Surface water	Primary	LOQ = 0.05 µg/L (Acetamiprid)	LC-QQQ	Norris D., 2017, DNA4037

Component of residue definition: acetamiprid (and metabolite IM-1-5)				
Matrix type	**Method type**	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Drinking water	ILV	LOQ = 0.05 µg/L (Acetamiprid)	LC-MS/MS	Eichler M., Herrmann S., 2018, 133112101
Drinking water	Primary	LOQ = 0.05 µg/L (IM-1-5)	LC-QQQ	Norris D., 2017, DNA4518
Drinking water	ILV	LOQ = 0.05 µg/L (IM-1-5)	LC-MS/MS	Eichler M., Herrmann S., 2018, 133141101

All analytical methods are active substance data and were evaluated during the EU review of acetamiprid. No additional studies have been performed.
These data have been provided and are considered to adequate.

5.3.2.6 Description of methods for the analysis of air (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in air is given in the following tables.

Table 5.3-8: Validated methods for air (if appropriate)

Component of residue definition: acetamiprid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	LOQ < 37 µg/m^3	HPLC-MS/MS	DAR 2001 Eguchi Y., 1997, NCAS 97-009
Primary	0.002 µg/m3	UPLC-MS/MS	Longhi D., 2019, GLP-STUDY-18-000080

All analytical methods are active substance data and were evaluated during the EU review of acetamiprid. No additional studies have been performed.
These data have been provided and are considered to adequate.

5.3.2.7 Description of methods for the analysis of body fluids and tissues (KCP 5.2)

An overview on the acceptable methods and possible data gaps for analysis of acetamiprid in body fluids and tissues is given in the following table.

Table 5.3-9: Methods for body fluids and tissues (if appropriate)

Component of residue definition: acetamiprid			
Method type	**Method LOQ**	**Principle of method (i.e. GC-MS or HPLC-UV)**	**Author(s), year / missing**
Primary	0.05 mg/l for blood 0.01 mg/kg for tissues	LC-MS/MS	xxx 2003, NCAS 03-235
Primary	0.05 mg/l for blood	UPLC-MS/MS	xxx., 2019, GLP-STUDY-18-000079

All analytical methods are active substance data and were evaluated during the EU review of acetamiprid. No additional studies have been performed.
These data have been provided and are considered to adequate for any special comments or remark.

5.3.2.8 Other studies/ information

No other studies have been provided

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	Owner
KCP 5.1.1	E. Arévalo	2019	A-200SL-OR3-C, Part I: Determination of physicochemical properties of the initial preparation, after accelerated storage BF-24/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry non GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/01	A. Markowicz	2019	Determination of residues of acetamiprid in/on maize under open field conditions following one application of A-200SL-OR3-CPD in Northern Europe in 2018 ZBBZ-2018/04/DPL/2 Food Safety Laboratory, Research Institute of Horticulture GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/02	A. Markowicz	2019	Determination of residues of acetamiprid in/on winter/spring oilseed rape under open field conditions following one and two applications of A-200SL-OR3-CPD in Northern Europe in 2018 ZBBZ-2018/04/DPL/1 Food Safety Laboratory, Research Institute of Horticulture GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/03	A. Markowicz	2020	Determination of residues of acetamiprid in/on oilseed rape under open field conditions following two applications of A-200SL-OR3-CPD in Northern Europe in 2019 428SRPL19R01 Food Safety Laboratory, Research Institute of Horticulture GLP	N	CIECH Sarzyna S.A.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			Unpublished		
KCP 5.1.2/04	A. Markowicz	2020	Determination of residues of acetamiprid in/on winter wheat under open field conditions following one applications of A-200SL-OR3-CPD in Northern Europe in 2019 428SRPL19R02 Food Safety Laboratory, Research Institute of Horticulture GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/05	E. Kulec-Płoszczyca	2019	A-200SL-OR3-C, Daphnia magna, Acute immobilisation test W/01/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/06	P. Bąk	2019	A-200SL-OR3-C, Chironomus sp., Acute immobilisation test W/02/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/07	E. Kulec-Płoszczyca	2019	A-200SL-OR3-C, Raphidocelis subcapitata SAG 61.81 (formerly Pseudokirchneriella subcapitata), Growth inhibition test W/03/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/08	K. Brzozowska-Wojoczek	2019	A-200SL-OR3-C, Daphnia magna, Reproduction test W/04/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP	N	CIECH Sarzyna S.A.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
			Unpublished		
KCP 5.1.2/ 09	M. Wołany	2019	A-200SL-OR3-C, Terrestial Plant Test: Vegetative Vigour Test G/151/18 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/ 10	M. Wołany	2019	A-200SL-OR3-C, Terrestial Plant Test: Seedling Emergence and Seedling Growth Test G/152/18 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/ 11	M. Grzesica	2019	A-200SL-OR3-C, Honeybees *(Apis mellifera L.)*, Chronic Oral Toxicity Test B/13/19 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A.
KCP 5.1.2/ 12	P. Holewik	2021	A-200SL-OR3-C, Honeybees *(Apis mellifera L.)*, Larval Toxicity Test, Repeated Exposure B-56-21 Łukasiewicz Research Network – Institute of Industrial Organic Chemistry, Branch Pszczyna GLP Unpublished	N	CIECH Sarzyna S.A

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	Owner
KCP 5.2/01	Longhi D.	2019	Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices, Final Report n° GLP-STUDY-18-000081, LabAnalysis s.r.l. GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/02	Ticco S. P.,	2019	Independent Laboratory Validation (ILV) of the analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices, CH - 031/2019, ChemService S.r.l, GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/03	xxx	2017	Validation of the Methods of Analysis used for the determination of acetamiprid and a specified metabolite in animal commodities, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99 Study Number: DNA4036, xxx Ltd. GLP Unpublished	N	PUH CHEMIROL Sp z o.o.
KCP 5.2/04	xxx.	2018	Acetamiprid and its metabolite IM-2-1: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Commodities, Study No. 133111101, Ibacon, GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	Owner
KCP 5.2/05	Norris, D	2018	Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1., Study code: DNA4517, David Norris Analytical Laboratories Ltd. GLP Unpublished	N	PUH CHEMIROL Sp z o.o.
KCP 5.2/06	Eichler, M. Herrmann S.	2018	Acetamiprid and its Metabolites IM-1-2 and IM-1-4: Independent Laboratory Validation of an Analytical Method for the Determination in Calcareous Soil Study No. 133113101, Ibacon, GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/07	Norris, D	2017	Validation of the Methods of Analysis used for the determination of acetamiprid in water, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99 Study Number: DNA4037, David Norris Analytical Laboratories Ltd. GLP Unpublished	N	PUH CHEMIROL Sp z o.o.
KCP 5.2/08	Norris, D	2018	Validation of the Methods of Analysis used for the Determination of a Metabolite of Acetamiprid in Drinking Water, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1 Study code: DNA4518, David Norris Analytical Laboratories Ltd. GLP Unpublished	N	PUH CHEMIROL Sp z o.o.
KCP 5.2/09	Eichler, M.	2018	Acetamiprid: Independent Laboratory Validation of an Analytical Method for the Determination in	N	PUH

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study Y/N**	**Owner**
	Herrmann S.		Drinking Water, Study No. 133112101, Ibacon, GLP Unpublished		CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/10	Eichler, M. Herrmann S.	2018	IM-1-5 (Metabolite of Acetamiprid): Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water Study No. 133141101, Ibacon, GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/11	Longhi D.	2019	Validation of an analytical method for the determination of Acetamiprid residues in air, GLP-STUDY-18-000080, LabAnalysis s.r.l. GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.
KCP 5.2/12	xxx.	2019	Validation of an analytical method for the determination of Acetamiprid residues in blood, GLP-STUDY-18-000079, LabAnalysis s.r.l. GLP Unpublished	N	PUH CHEMIROL Sp z o.o., PROPLAN, EXCLUSIVAS SARABIA, S.A.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Verte-brate study Y/N	Owner
KCP 5.2	Tokieda M.	1997	Analytical method for the determination of acetamiprid in water (validation study) Nisso Chemical Analysis Service Co. NCAS 97-007 GLP Unpublished	N	NPS
KCP 5.2	Yang J.	1998	Acetamiprid (NI-25), Validation of Methods of Analysis for NI-25 and its metabolites, IC-0, IM-1-4, IM-1-2, in soil using LC/MS/MS Rhone-Poulenc Agriculture EC-97-411 GLP Unpublished	N	NPS
KCP 5.2	Neal J.L.	2003	Acetamiprid: Validation of the method of analysis for the determination of residues of the acetamiprid metabolites AE 0653700 (IM-1-5) residues in calcareous soils by LC/MS/MS Bayer CropScience 02Y536660 GLP Unpublished	N	BAY
KCP 5.2	Eguchi Y.	1997	Analytical method for the determination of acetamiprid in air Nisso Chemical Analysis Service Co. NCAS 97-009 GLP Unpublished	N	NPS
KCP 5.2	xxx.	1997	Acetamiprid and its metabolites IM-2-1: Analytical method for the determination of residues in foodstuff of ruminant origin (milk, muscle, fat, liver and kidney) Project No 660216 GLP Unpublished	Y	ROP

KCP 5.2	xxx	1997	Acetamiprid and its metabolites IM-2-1: Analytical method for the determination of residues in foodstuff of hen origin (egg, muscle, fat and liver) Project No 660227 GLP Unpublished	Y	ROP
KCP 5.2	Fuchsbichler G.	2000	Validation of the multi residue method (DFG S19 modified) for the determination of acetamiprid in apples and tomatoes Bayerische Hauptversuchanstalt fur Landwirtschaft HVA 21/00 GLP Unpublished	N	Aventis & NPS
KCP 5.2	Williams M.	1999	Validation of Residue Analytical Metfod of Insecticide Acetamiprid (NI-25) in Crops-Parent Method (Nippon Repont No. EC-521) in/on Various Agricultural Crop Substrates Horizon Laboratories Inc., Analytical BioChemistry Laboratories Inc. EC-97-388 GLP Unpublished	N	Aventis & NPS
KCP 5.2	Communal P.Y.	1997	Validation of the assay method relative to the residues of acetamiprid (NI-25) in tobacco, cotton, pepper, pear, peach, orange and apple samples ADME Bioanalyses RPA/NI-25/95101N GLP Unpublished	N	ROP
KCP 5.2	Goller G.	1998	Validation of acetamiprid (NI-25) observed in tomato, cucumber, plum and melon samples ADME Bioanalyses RPA/NI-25/97062 GLP Unpublished	N	ROP

KCP 5.2	xxx	2003	Development and Validation of the Analytical Method for the Determination of Acetamiprid in Body Fluids and Tissues Nisso Chemical Analysis Cervice Co., Ltd. Nippon Soda Co., Ltd. Study No.: NCAS 03-235 GLP Unpublished	N	NPS

Appendix 2 Detailed evaluation of submitted analytical methods

A 2.1 Analytical methods for acetamiprid

A 2.1.1 Methods used for the generation of pre-authorization data (KCP 5.1)

A 2.1.1.1 Determination of residues of acetamiprid in maize

A 2.1.1.1.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-MS/MS method was used to determine acetamiprid in maize. Two mass transitions were applied. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.01 mg/kg and 0.5 mg/kg for both ion mass transitions of acetamiprid were all in the range of 70 – 110 % consistently with the standard acceptance criteria. All precision values at the fortification levels of 0.01 mg/kg and 0.5 mg/kg for both ion mass transitions were < 20 %. The LOQ was established at 0.01 mg/kg. The interfering signals in control specimen were negligible. It is concluded that the analytical method was shown to be highly selective and suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	Determination of residues of acetamiprid in/on maize under open field conditions following one applicdation of A-200SL-OR3-CPD in Northern Europe in 2018, A. Markowicz, 2019, ZBBZ-2018/04/DPL/2
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In brief, samples of maize were extracted with acetonitrile after addition of water. After addition of a buffer-salt mixture containing magnesium sulfate, sodium chloride and sodium citrate the extract was shaken. Following centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulfate addition.

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The following chromatographic conditions were used:

- Oven temperature 40 ºC
- Mobile phase flow v = 0.4 mL/min
- Injection volume 10 µL

- Autosampler temperature 10 ºC
- Mobile phase
  Eluent A: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water
  Eluent B: 5 mM Ammonium Formate with 0.01% (v/v) Formic acid in Acetonitrile:Water, 95:5
- Post time 6 min
- Total analysis time 20 min
- Retention time (approx..) 7.48 min

Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Confirmation ion ratio for Acetamiprid in all samples were within ± 30 % of the average found for the standards.
No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for maize matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Results and discussions

Table A 1: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Overall Mean Rec. (%)	Overall RSD (%)	Comments
Quantification Ion Mass Transition m/z 223.1→126.1							
Maize (kernels)	Acetamiprid	0.01	92	2.6	93	2.1	-
		0.5	93	1.8			
Confirmation Ion Mass Transition m/z 223.1→73.1							
Maize (kernels)	Acetamiprid	0.01	91	4.7	92	3.4	-
		0.5	93	1.4			

Table A 2: Characteristics for the analytical method used for validation of acetamiprid residues in maize (kernels)

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
Calibration (type, number of data points)	Linear equation: y = 2611885.459936x + 186.690412 (quantification) y= 649709.642707x + 55.643380 (confirmation)
Calibration range	The calibration was performed using calibration solutions (9 concentrations) within the range of 0.0002 to 0.1 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The limit of quantification (LOQ) was successfully established at 0.01

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
	mg/kg The limit of detection (LOD) for Acetamiprid was set at 0.002 mg/kg

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and < 20% RSD.
No interferences at the retention time of acetamiprid above 30% of the LOQ (limit of quantification) were observed in the control matrices.
A highly specific detection system was used (MS/MS).
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.1.1.1.1 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in plant sample is specific for acetamiprid. For details please see point A 2.1.1.1.1.

A 2.1.1.2 Determination of residues of acetamiprid in oilseed rape

A 2.1.1.2.1 Method validation

Comments of zRMS:	The validation of the analytical method has been accepted. The general principles of the analytical procedure for acetamiprid in OSR were based on the normalized method EN 15662:2008. The requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 were met. This LC-MS/MS method employs two selected ion mass transitions for quantitation and confirmation. The mean recovery values at the fortification levels of 0.01 mg/kg and 0.5 mg/kg for both ion mass transitions were all in the range 70 – 110 %, all RSD < 20%. The method fulfils the requirements regarding specificity, linearity, repeatability, LOQ and recoveries and is therefore suitable to the assigned purposes.

Reference:	KCP 5.1.2
Report	Determination of residues of acetamiprid in/on winter/spring oilseed rape under open field conditions following one and two applications of A-200SL-OR3-CPD in Northern Europe in 2018, A. Markowicz, 2019, ZBBZ-2018/04/DPL/1
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1)

Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In brief, samples of Oilseed rape (OSR) were extracted with acetonitrile after addition of water. After addition of a buffer-salt mixture containing magnesium sulfate, sodium chloride and sodium citrate the extract was shaken. Following centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA), silica sorbent (C18EC) and dehydrated by magnesium sulfate addition.

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The following chromatographic conditions were used:

- Oven temperature 40 ºC
- Mobile phase flow v = 0.4 mL/min
- Injection volume 10 µL
- Autosampler temperature 10 ºC
- Mobile phase
  Eluent A: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water
  Eluent B: 5 mM Ammonium Formate with 0.01% (v/v) Formic acid in Acetonitrile:Water, 95:5
- Post time 6 min
- Total analysis time 20 min
- Retention time (approx..) 7.48 min

Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Confirmation ion ratio for Acetamiprid in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Results and discussions

Table A 3: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Overall Mean Rec. (%)	Overall RSD (%)	Comments
Quantification Ion Mass Transition m/z 223.1→126.1							
Oilseed rape (seeds)	Acetamiprid	0.01	91	4.0	93	3.9	-
		0.5	96	1.5			
Confirmation Ion Mass Transition m/z 223.1→73.1							
Oilseed rape	Acetamiprid	0.01	92	3.5	94	3.5	-

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Overall Mean Rec. (%)	Overall RSD (%)	Comments
(seeds)		0.5	96	1.4			

Table A 4: Characteristics for the analytical method used for validation of acetamiprid residues in oilseed rape (seeds)

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
Calibration (type, number of data points)	Linear equation: y = 2715103.301276x + 60.556339 (quantification) y = 669427.017488x + 5.124644 (confirmation)
Calibration range	The calibration was performed using calibration solutions (9 concentrations) within the range of 0.0002 to 0.1 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The limit of quantification (LOQ) was successfully established at 0.01 mg/kg The limit of detection (LOD) for Acetamiprid was set at 0.002 mg/kg

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and < 20% RSD.
No interferences at the retention time of acetamiprid above 30% of the LOQ (limit of quantification) were observed in the control matrices.
A highly specific detection system was used (MS/MS).
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.1.2.2 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in plant sample is specific for acetamiprid. For details please see point A 2.1.1.2.1.

A 2.1.1.2.3 Method validation

Comments of zRMS:	The validation of the analytical method has been accepted. The study objective was to determine residues of acetamiprid in oilseed rape seeds after two applications of the subjected PPP. The requirements of SANCO/825/00 rev. 8.1 and SANCO/3029/99 rev. 4 were met. This LC-MS/MS method employs two selected ion mass transitions for quantitation and confirmation. The mean recovery values at the fortification levels of 0.01 mg/kg (LOQ) and 0.1 mg/kg for both ion mass transitions were all in the range 70 – 110 %, all RSD < 20%. The method fulfils the requirements regarding specificity, linearity, repeatability, LOQ and recoveries and is therefore suitable to the assigned purposes.

Reference:	KCP 5.1.2
Report	Determination of residues of acetamiprid in/on oilseed rape under open field conditions following two applications of A-200SL-OR3-CPD in Northern Europe in 2019, A. Markowicz, 2020, 428SRPL19R01
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In brief, samples of Oilseed rape (OSR) were extracted with acetonitrile after addition of water. After addition of a buffer-salt mixture containing magnesium sulfate, sodium chloride and sodium citrate the extract was shaken. Following centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA), silica sorbent (C18EC) and dehydrated by magnesium sulfate addition.

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The following chromatographic conditions were used:

- Oven temperature 40 ºC
- Mobile phase flow v = 0.4 mL/min
- Injection volume 10 µL
- Autosampler temperature 10 ºC
- Mobile phase
  Eluent A: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water
  Eluent B: 5 mM Ammonium Formate with 0.01% (v/v) Formic acid in Acetonitrile:Water, 95:5
- Post time 6 min
- Total analysis time 20 min
- Retention time (approx..) 6.25 min

Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Confirmation ion ratio for Acetamiprid in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for oilseed rape matrix, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Results and discussions

Table A 5: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 3)	Mean recovery (%)	RSD (%)	Overall Mean Rec. (%)	Overall RSD (%)	Comments
Quantification Ion Mass Transition m/z 223.1→126.1							
Oilseed rape (seeds)	Acetamiprid	0.01	94	1.9	91	4.1	-
		0.1	88	1.1			
Confirmation Ion Mass Transition m/z 223.1→73.1							
Oilseed rape (seeds)	Acetamiprid	0.01	98	1.8	93	6.1	-
		0.1	88	1.5			

Table A 6: Characteristics for the analytical method used for validation of acetamiprid residues in oilseed rape (seeds)

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
Calibration (type, number of data points)	Linear equation: y = 6829990x – 633.023029 (quantification) y = 1640865.973602x – 120.712135 (confirmation)
Calibration range	The calibration was performed using calibration solutions (9 concentrations) within the range of 0.0002 to 0.1 µg/mL. The calibrations were found to be linear with correlation coefficients (r) greater than 0.998. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The limit of quantification (LOQ) was successfully established at 0.01 mg/kg The limit of detection (LOD) for Acetamiprid was set at 0.002 mg/kg

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and < 20% RSD.
No interferences at the retention time of acetamiprid above 30% of the LOQ (limit of quantification) were observed in the control matrices.
A highly specific detection system was used (MS/MS).
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.1.2.4 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in plant sample is specific for acetamiprid. For details please see point A 2.1.1.2.2.

A 2.1.1.3 Determination of residues of acetamiprid in wheat

A 2.1.1.3.1 Method validation

Comments of zRMS:	The validation of the analytical method has been accepted. The study objective was to determine residues of acetamiprid in wheat. This LC-MS/MS method employs two selected ion mass transitions for quantitation and confirmation. The mean recovery values at the fortification levels of 0.01 mg/kg and 0.5 mg/kg or 1 mg/kg for both ion mass transitions were all in the range 70 – 110 %, all RSD < 20%. The method fulfils the requirements regarding specificity, linearity, repeatability, LOQ and recoveries and is therefore suitable to the assigned purposes.

Reference:	KCP 5.1.2
Report	Determination of residues of acetamiprid in/on winter wheat under open field conditions following one applications of A-200SL-OR3-CPD in Northern Europe in 2019, A. Markowicz, 2020, 428SRPL19R02
Guideline(s):	Yes (SANCO/3029/99 rev. 4, SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

In brief, samples of wheat were extracted with acetonitrile after addition of water. After addition of a buffer-salt mixture containing magnesium sulfate, sodium chloride and sodium citrate the extract was shaken. Following centrifugation, an aliquot of the upper acetonitrile phase was cleaned by primary secondary amine (PSA) and dehydrated by magnesium sulfate addition.

Quantification was performed by use of highly selective liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The following chromatographic conditions were used:

- Oven temperature 40 ºC
- Mobile phase flow v = 0.4 mL/min
- Injection volume 10 µL
- Autosampler temperature 10 ºC
- Mobile phase
  Eluent A: 5 mM Ammonium Formate with 0.01% (v/v) Formic Acid in Water
  Eluent B: 5 mM Ammonium Formate with 0.01% (v/v) Formic acid in Acetonitrile:Water, 95:5

- Post time 6 min
- Total analysis time 20 min
- Retention time (approx..) 6.25 min

Two selected ion mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. The retention time of analyte in extracts corresponds to that of the calibration standards with a tolerance of < ± 0.1 min. Confirmation ion ratio for Acetamiprid in all samples were within ± 30 % of the average found for the standards.

No significant interference above 30 % of LOQ was detected in any of the reagent blanks or control specimen extracts for wheat matrices, so that a highly level of selectivity was demonstrated and an additional confirmatory method is not necessary.

Results and discussions

Table A 7: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Overall Mean Rec. (%)	Overall RSD (%)	Comments
Quantification Ion Mass Transition m/z 223.1→126.1							
Wheat grain	Acetamiprid	0.01	92	1.9	91	2.8	-
		0.5	90	3.1			
Wheat straw	Acetamiprid	0.01	95	4.6	92	5.0	-
		1	90	4.0			
Confirmation Ion Mass Transition m/z 223.1→56.1							
Wheat grain	Acetamiprid	0.01	93	2.9	92	3.2	-
		0.5	90	2.8			
Wheat straw	Acetamiprid	0.01	96	5.4	93	5.5	-
		1	90	4.1			

Table A 8: Characteristics for the analytical method used for validation of acetamiprid residues in wheat grain and straw

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
Calibration (type, number of data points)	Linear equations: Wheat grain y = 9819307x – 288.485307 (quantification) y = 7732278x – 188.324901 (confirmation) Wheat straw y = 4375044.946752x – 213.448595 (quantification) y = 3431787.802377x – 191.353709 (confirmation)

	Acetamiprid
Specificity	A highly specific detection system was used (MS/MS) blank value < 30 % LOQ The method is specific for Acetamiprid
Calibration range	The linearity of the detector response for Acetamiprid was demonstrated by single determination of matrix-matched calibration standards at nine concentration levels ranging from 0.0002 µg/mL to 0.1 µg/mL for wheat grain and at ten concentration levels ranging from 0.0001 µg/mL to 0.1 µg/mL for wheat straw. These ranges correspond from 0.002 mg/kg to 1 mg/kg for wheat grain and from 0.002 mg/kg to 2 mg/kg for wheat straw thus covering the range from no more than 30 % of the LOQ and at least + 20 % of the highest analyte concentration level detected in the sample. The calibration curves obtained for both ion mass transitions of Acetamiprid were linear with the coefficients of correlation (R) greater than 0.99. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	yes
Limit of determination/quantification	The limit of quantification (LOQ) was successfully established at 0.01 mg/kg The limit of detection (LOD) for Acetamiprid was set at 0.002 mg/kg

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 120% for mean recovery and < 20% RSD.
No interferences at the retention time of acetamiprid above 30% of the LOQ (limit of quantification) were observed in the control matrices.
A highly specific detection system was used (MS/MS).
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.1.3.2 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in plant sample is specific for acetamiprid. For details please see point A 2.1.1.3.1.

A 2.1.1.4 Determination of acetamiprid in Elendt M7 medium in support of ecotoxicological studies. Analysis of acetamiprid in acute immobilization test in *Daphnia magna* and *Chironomus* sp. Analysis acetamiprid in reproduction test in *Daphnia magna.*

A 2.1.1.4.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in Elendt M7 medium. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.0005 mg/L (LOQ) and 0.05 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity (8 point calibration). It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Daphnia magna, Acute immobilisation test, E. Kulec-Płoszczyca, 2019, W/01/19
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was developed for the determination of acetamiprid in Elendt M7 medium. The range of linearity of the analytical graphs, specificity, precision, recovery, and limits of quantification and detection of analytes were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection.
The following chromatographic conditions were used:

- Chromatographic System: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Shimadzu, Prominence-i (Shimadzu Corporation Japan)
- Analytical Column: Gemini NX 3µ C18 100A, l = 150 mm, Ø = 4.6 mm
- Oven temperature: 35°C
- Injection Volume: 20 µl
- Mobile Phase: acetonitrile for HPLC : 0.05% ortho-phosphoric acid (35:65, v/v)
- Flow Rate: 0.5 ml/min
- Wave length: 246 nm
- Detection System: Diode Array Detector

Results and discussions

Table A 9: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Elendt M7 medium	Acetamiprid	0.0005	91.9	2.71	-
		0.05	98.7	0.42	

Table A 10: Characteristics for the analytical method used for validation of acetamiprid residues in Elendt M7 medium

	Acetamiprid
Specificity	The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control Elendt M7 medium samples, and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference is directly apparent and would be observed in the chromatogram of he matrix control sample.
Calibration (type, number of data points)	Linear equation: y = 201436x + 397.900 R= 0.9997975
Calibration range	Working solutions of acetamiprid at the concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graph range from 0.01 µg/mL to 10 µg/mL. The volumes of 20 µL were injected for standards solution. The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of Quantification (LoQ) for acetamiprid analyzed in Elendt M7 medium is 0.0005 mg/L. Limit of Detection (LoD) is 0.00015 mg/L.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

A 2.1.1.4.2 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in Elendt M7 medium is specific for acetamiprid.
For details please see point A 2.1.1.4.1.

A 2.1.1.4.3 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in Elendt M7 medium. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.0005 mg/L (LOQ) and 0.05 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Chironomus sp., Acute immobilisation test, P. Bąk, 2019, W/02/19
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For method validation details see point A 2.1.1.4.1.

A 2.1.1.4.4 Confirmatory method

See point A 2.1.1.4.1.1

Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in Elendt M7 medium. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.0005 mg/L (LOQ) and 0.05 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Daphnia magna, Reproduction test, K. Brzozowska-Wojoczek, 2019, W/04/19
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No

GLP:	Yes
Acceptability:	Yes

For method validation details see point A 2.1.1.4.1.

Confirmatory method

See point A 2.1.1.4.1.1

A 2.1.1.5 Determination of acetamiprid in AAP medium in support of ecotoxicological studies. Analysis of acetamiprid in growth inhibition test in Pseudokirchneriella subcapitata.

A 2.1.1.5.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in AAP. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.0005 mg/L (LOQ) and 0.05 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SLOR3-C, Raphidocelis subcapitata SAG 61.81(formerly Pseudokirchneriella subcapitata) growth inhibition test, E. Kulec-Płoszczyca, 2019, W/03/19
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was developed for the determination of acetamiprid in AAP medium. The range of linearity of the analytical graphs, specificity, precision, recovery, and limits of quantification and detection of analytes were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection.
The following chromatographic conditions were used:

- Chromatographic System: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Shimadzu, Prominence-i (Shimadzu Corporation Japan)

- Analytical Column: Gemini NX 3µ C18 100A, l = 150 mm, Ø = 4.6 mm
- Oven temperature: 35°C
- Injection Volume: 20 µl
- Mobile Phase: acetonitrile for HPLC : 0.05% ortho-phosphoric acid (35:65, v/v)
- Flow Rate: 0.5 ml/min
- Wave length: 246 nm
- Detection System: Diode Array Detector

Results and discussions

Table A 11: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
AAP medium	Acetamiprid	0.0005	100.0	1.14	-
		0.05	102.4	0.44	

Table A 12: Characteristics for the analytical method used for validation of acetamiprid residues in AAP medium

	Acetamiprid
Specificity	The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control AAP medium samples, and fortified samples. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference is directly apparent and would be observed in the chromatogram of the matrix control sample.
Calibration (type, number of data points)	Linear equation: y = 201436x + 397.900 R= 0.9997975
Calibration range	Working solutions of acetamiprid at the concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graphs range from 0.01 µg/mL to 10 µg/mL. The volumes of 20 µL were injected for standards solution The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of Quantification (LoQ) for acetamiprid analyzed in AAP medium is 0.0005 mg/L. Limit of Detection (LoD) is 0.00015 mg/L.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

A 2.1.1.5.2 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in AAP medium is specific for acetamiprid. For details please see point A 2.1.1.5.1.

A 2.1.1.6 Determination of acetamiprid in water in support of ecotoxicological studies. Analysis of acetamiprid in Vegetative Vigour test.

A 2.1.1.6.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in water. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.1 mg/L (LOQ) and 10 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Terrestial Plant Test: Vegetative Vigour Test, M. Wołany, 2019, G/151/18
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was developed for the determination of acetamiprid in water. The range of linearity of the analytical graphs, specificity, precision, recovery, and limits of quantification and detection of analytes were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. Prior to analysis, the samples were diluted.
The following chromatographic conditions were used:

- Chromatographic System: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Shimadzu, Prominence-i (Shimadzu Corporation Japan)
- Analytical Column: Gemini NX 3µ C18 100A, l = 150 mm, Ø = 4.6 mm
- Oven temperature: 35°C
- Injection Volume: 20 µl
- Mobile Phase: acetonitrile for HPLC : 0.05% ortho-phosphoric acid (35:65, v/v)

- Flow Rate: 0.5 ml/min
- Wave length: 246 nm
- Detection System: Diode Array Detector

Results and discussions

Table A 13: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.1	101.2	0.3	-
		10.0	104.4	0.1	

Table A 14: Characteristics for the analytical method used for validation of acetamiprid residues in water.

	Acetamiprid
Specificity	The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control water samples, and fortified samples of matrices. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference is directly apparent and would be observed in the chromatogram of the matrix control sample.
Calibration (type, number of data points)	Linear equation: y = 201436x + 397.900 R= 0.9997975
Calibration range	Working solutions of acetamiprid at the concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graphs range from 0.01 µg/mL to 10 µg/mL. The volumes of 20 µL were injected for standards solution. The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of Quantification (LoQ) for acetamiprid analyzed in water phase is 0.1 mg/L. Limit of Detection (LoD) is 0.03 mg/L.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

A 2.1.1.6.2 Confirmatory method

No confirmatory method is required .
According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where

primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in water is specific for acetamiprid. For details please see point A 2.1.1.9.1.

A 2.1.1.6.3 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in water phase. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.1 mg/L (LOQ) and 10 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Terrestial Plant Test: Seedling Emergence and Seedling Growth Test, M. Wołany, 2019, G/152/18
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

For method validation details see p. A 2.1.1.6.1

A 2.1.1.6.4 Confirmatory method

See p. A 2.1.1.9.1.1

A 2.1.1.7 Determination of acetamiprid in water and sucrose solution in support of ecotoxicological studies. Analysis of acetamiprid in Chronic Oral Toxicity Test.

A 2.1.1.7.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in water and sucrose solution. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.1 mg/L and 10 mg/L as well as of 0.2 mg/L and 2 mg/L were all in the range of 70 – 110 % (RSD < 10%) consistently with the standard acceptance criteria of SANCO/3029/99 rev. 4. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ set in water is 0,1 mg/L, in sucrose sol. 0,2 mg/L. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Honeybees (Apis mellifera L.), Chronic Oral Toxicity Test, M. Grzesica, 2019, B/13/19
Guideline(s):	Yes (SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was developed for the determination of acetamiprid in water and sucrose solution. The range of linearity of the analytical graphs, specificity, precision, recovery, and limits of quantification and detection of analytes were determined.
The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. Prior to analysis, the samples were using solid phase extraction cartridges for water phase.
The following chromatographic conditions were used:

- Chromatographic System: High Performance Liquid Chromatography (HPLC)
- Chromatograph: Shimadzu, Prominence-i (Shimadzu Corporation Japan)
- Analytical Column: Gemini NX 3µ C18 100A, l = 150 mm, Ø = 4.6 mm
- Oven temperature: 35°C
- Injection Volume: 20 µl
- Mobile Phase: acetonitrile for HPLC : 0.05% ortho-phosphoric acid (35:65, v/v)
- Flow Rate: 0.5 ml/min
- Wave length: 246 nm
- Detection System: Diode Array Detector

Results and discussions

Table A 15: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.1	101.2	0.3	-
		10.0	104.4	0.1	
Sucrose solution	Acetamiprid	0.2	97.6	3.9	
		2.0	102.5	1.5	

Table A 16: Characteristics for the analytical method used for validation of acetamiprid residues in water and sucrose solution.

	Acetamiprid
Specificity	The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control water samples, and fortified samples of matrices. Considering the results of the analysis, no signal of detected substance was overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated. Furthermore, any interference is directly apparent and would be observed in the chromatogram of the matrix control sample.
Calibration (type, number of data points)	Linear equation: y = 201436x + 397.900 R= 0.9997975
Calibration range	Working solutions of acetamiprid at the concentrations of 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0 and 10.0 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of linearity of the analytical graphs range from 0.01 µg/mL to 10 µg/mL. The volumes of 20 µL were injected for standards solution. The calibrations were found to be linear with correlation coefficients (r) greater than 0.999. These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	Limit of Quantification (LoQ) for acetamipri is 0.1 mg/L and Limit of Detection (LoD) is 0.03 mg/L in water. Limit of Quantification (LoQ) for acetamipri is 0.2 mg/L and Limit of Detection (LoD) is 0.06 mg/L in sucrose solution.

Conclusion

The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4

A 2.1.1.7.2 Confirmatory method

No confirmatory method is required .

According to SANCO/3029/99 rev. 4 11/07/00 additional confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in water and sucrose solution is specific for acetamiprid. For details please see point A 2.1.1.8.1.

A 2.1.1.8 Determination of acetamiprid in water in support of ecotoxicological studies. Analysis of acetamiprid in Larval Toxicity Test, Repeated Exposure.

A 2.1.1.8.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-DAD method was used to determine concentrations of acetamiprid in water. Five recovery determinations were performed at two fortification levels. The mean recovery values at the fortification level of 0.06 mg/L and 0,6 mg/L were all in the range of 70 – 110 % (RSD < 10%). The analytical method was performed according to SANTE/2020/12830, Rev. 1. of February 24, 2021, Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes that supersedes Guidance Documents SANCO/3029/99 and SANCO/825/00. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ set in water is 0,06 mg/L. Assessment of matrix effects was performed. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.1.2
Report	A-200SL-OR3-C, Honeybees (Apis mellifera L.), Larval Toxicity Test, Repeated Exposure, P. Holewik, 2021, B-56-21
Guideline(s):	SANTE/2020/12830, Rev. 1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The analytical method was developed for the determination of acetamiprid in water. The determination was accomplished by the high performance liquid chromatography (HPLC) with DAD detection. The chromatographic systems and conditions used for the analysis of acetamiprid are shown below.

	Parameter
Chromatographic System	High Performance Liquid Chromatography (HPLC)
Chromatograph	Shimadzu, Prominence-*i* (Shimadzu Corporation Japan)
Analytical Column	Luna 5µm C18(2) 100Å , l = 250 mm, Ø = 4.6 mm
Oven temperature	35ºC
Injection Volume	20 µL
Mobile Phase	acetonitrile for HPLC: 0.05% ortho-phosphoric acid (40:60,

	v/v)
Flow Rate	1.0 mL/min
Wave length	246 nm
Detection System	Diode Array Detector

Results and discussions

Table A 17: Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Water	Acetamiprid	0.06	96.7	2.0	-
		0.6	94.3	0.3	

Table A 18: Characteristics for the analytical method used for validation of acetamiprid residues in water.

Calibration data	Acetamiprid
Linearity	Working solutions of acetamiprid at the concentrations of 0.02 µg/mL, 0.05 µg/mL, 0.1 µg/mL, 0.2 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of the first linearity of the analytical graph of acetamiprid: 0.02 µg/mL to 1 µg/mL, y = 106575x + 50.8927, R^2 = 0.9998556 The range of the second linearity of the analytical graph of acetamiprid: 0.5 µg/mL to 10 µg/mL, y = 105225x + 829.651, R^2 = 0.9998877
Selectivity and specificity	The analytical method specificity was estimated on the basic of the analysis of the chromatograms obtained for the control matrix, and fortified samples of matrix. Considering the results of the analysis, no signal of detected substance is overlapping with matrix signal of the control samples in the experimental conditions. Therefore, the specificity of the method was demonstrated.
Precision	Precision of a particular method is defined as its repeatability (RSD – relative standard deviation [%]). The repeatability for detected substance analyzed in the water is from 0.3% to 2.0%. The precision is: - 2.0% at level 0.06 mg acetamiprid/L water - 0.3% at level 0.6 mg acetamiprid/L water.
Accuracy	The accuracy of the method is reported as mean recovery ± relative standard deviation. The mean recovery range for acetamiprid in water is from 94.3 ± 0.3% to 96.7 ± 2.0%.

Calibration data	Acetamiprid
Linearity	Working solutions of acetamiprid at the concentrations of 0.02 µg/mL, 0.05 µg/mL, 0.1 µg/mL, 0.2 µg/mL, 0.5 µg/mL, 1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL were injected successively to the chromatographic column and the chromatograms were recorded. The range of the first linearity of the analytical graph of acetamiprid: 0.02 µg/mL to 1 µg/mL, y = 106575x + 50.8927, R^2 = 0.9998556 The range of the second linearity of the analytical graph of acetamiprid: 0.5 µg/mL to 10 µg/mL, y = 105225x + 829.651, R^2 = 0.9998877
Matrix Effect	Assessment of matrix effects was performed by comparing the analyte response of one individual standard at concentration 0.03 µg acetamiprid/mL prepared in solvent to at concentration 0.03 µg acetamiprid/mL prepared in blank matrix of water. Matrix effect for acetamiprid is -5.0 % and not exceed ±20%.
Limit of Quantification	Limit of Quantification was estimated as the lowest concentration of a detected substance at which an acceptable mean recovery is obtained (normally 70 – 120% with a relative standard deviation of preferably ≤ 20%). LOQ = 0.06 mg acetamiprid/L water
Limit of Detection	The limit of detection (LoD) is defined as the lowest detectable concentration or amount of an analyte in a sample. LOD = 0.04 mg acetamiprid/L water
Stability	The stability of stock solution of acetamirpid was tested at concentrations 1000 mg acetamiprid/L. Data for stability were obtained after 0 day, 6 days and 21 days of storage at cool temperature i.e. 2^0C – 8^0C. Compared to the mean recoveries measured at 0 day, significant decline was not observed after 21 days.

Days of storage	Mean recovery ± RSD[%]
0	105.3 ± 0.4
6	105.8 ± 0.9
12	103.8 ± 0.5

Conclusion

The analytical method therefore meets the requirements of guideline SANTE/2020/12830, Rev. 1.

A 2.1.1.8.2 Confirmatory method

No confirmatory method is required .
According to SANTE/2020/12830, Rev. 1 confirmatory analysis will not be required where primary residue method is shown to be specific to the analyte of interest.
The method which was used to determination of residues in water is specific for acetamiprid.

Methods for post-authorization control and monitoring purposes (KCP 5.2)

A 2.1.2.1 Description of analytical methods for the determination of residues in plant matrices (KCP 5.2)

A 2.1.2.1.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical UPLC-MS/MS method for determination of acetamiprid was successfully validated in oranges, sunflower seeds and maize grain. Two mass transitions were applied. Five recovery determinations were performed at two fortification levels for each matrix. The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recoveries values in the range of 70-110% with RSD% < 20% at each fortification level for each matrix. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set in each matrix at 0,01. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices, D. Longhi, 2019, GLP-STUDY-18-000081
Guideline(s):	Yes (SANCO/825/00, rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of this Study was the Validation of an analytical method for the determination of Acetamiprid in high acid content, high oil content and dry/high starch content matrices (oranges, sunflower seeds and maize grain). The validated method consisted in an extraction of the analyte from the matrices with acetonitrile under ultrasonic conditions. After extraction, water was added to the extracts, followed by a QuEChERS salts mixture. After centrifugation, an aliquot of the organic supernatant was filtered an injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and
m/z 223 to m/z 56 (secondary confirmation detection 223/56). The applied analytical method was validated under GLP compliance according to the SANCO/825/00 rev.8.1 guideline.

Analysis was performed under following chromatographic and mass spectrometric conditions:

Instrument	Agilent UPLC 1290 Infinity II + mass spectrometer Agilent 6470;
Column	Zorbax Eclipse Plus C18, 1.8 µm × 2.1 mm × 50 mm;

Column temperature	40 °C
Injection volume	2 µL
Mobile phases	A: 0.2 % v/v formic acid in Q1 water (prepared dissolving a volume of 2 mL of formic acid in 1 L of Q1 water); - Mobile phase B: 0.1 % v/v formic acid in methanol (prepared dissolving a volume of 1 mL of formic acid in 1 L of methanol for UPLC/MS);
Gradient	<table><tr><th>Time (min)</th><th>% A</th><th>% B</th></tr><tr><td>0</td><td>95</td><td>5</td></tr><tr><td>1</td><td>95</td><td>5</td></tr><tr><td>4</td><td>5</td><td>95</td></tr><tr><td>6</td><td>5</td><td>95</td></tr></table>
Flow	0.4 mL/min;
Retention time	3.0 min;
Ionisation type	Electrospray ionisation (ESI)
Polarity	Positive ion mode

Results and discussions

Table A 17 Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Primary transition 223/126					
Orange	Acetamiprid	0.01	92.5	2.0	
		0.1	97	4.4	
Sunflower seeds		0.01	95.8	2.1	
		0.1	91.7	0.5	
Maize grain		0.01	102.9	0.8	
		0.1	101.3	1.2	
Confirmatory transition 223/56					
Orange	Acetamiprid	0.01	92.5	1.7	
		0.1	94.7	2.3	
Sunflower seeds		0.01	95.5	1.7	
		0.1	91	0.5	
Maize grain		0.01	99.3	1.8	
		0.1	102.1	0.9	

Table A 18 Characteristics for the analytical method used for validation of acetamiprid residues in plant matrix

<table>
<tr><th></th><th>Acetamiprid</th></tr>
<tr><td>Specificity</td><td>Quantification was performed by use of LC-MS/MS detection. Two mass transitions were evaluated in order to demonstrate that the method achieves a high level of selectivity. No significant interference above 30 % of LOQ was detected in any of the reagent blanks or the control sample extracts of each matrix, so that a high level of selectivity was demonstrated.</td></tr>
<tr><td>Calibration (type, number of data points)</td><td>Linearity was checked by a 5-points calibration curve (single injection) using matrix matched standard solutions. All the obtained calibration curves had R^2 values in accordance with that prefixed ($R^2 > 0.99$). Here below the calibration curves, the graphs with the response factors versus the concentrations (with the x axis fixed on the mean response factor) and the graphs with the response factors relative to their mean versus the concentrations are reported.
<table>
<tr><td></td><td>Primary detection (MRM transition 223/126)</td><td>Confirmatory detection (MRM transition 223/56)</td></tr>
<tr><td>Orange</td><td>y = 1240.150675*x -207.608047</td><td>y = 605.058169*x – 85.196954</td></tr>
<tr><td>Sunflower seeds</td><td>y = 1120.768961*x – 174.085515</td><td>y = 558.319610*x – 87.890525</td></tr>
<tr><td>Maize grain</td><td>y = 616.722120*x – 183.460056</td><td>y = 299.145435*x – 47.207462</td></tr>
</table></td></tr>
<tr><td>Calibration range</td><td>The linearity was evaluated on the same calibration range from 1.041 to 52.05 µg/L (equal to from 4.16 to 208 µg/kg in the original samples) in 5 levels for each matrix, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56. These results meet the acceptance criteria of $r \geq 0.99$. The amounts of the analyte were calculated with the external standard method. 5 matrix matched standard solutions for each matrix were analysed in order to obtain a calibration curve interpolated with a linear regression calculated with the least squares method.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>yes</td></tr>
<tr><td>Limit of determination/quantification</td><td>The Limit of quantitation (LOQ) is defined as the lowest concentration at which an acceptable recovery is obtained. The target LOQ for this Study was set at 0.01 mg/kg. Limit of detection (LOD) is the smallest concentration at which the analyte produces an instrumental signal at least 3 times higher than the background noise of the chromatogram. It should be not higher than 30% of the LOQ value. The LOD was tested with a matrix matched standard solution of 0.521 µg/L (corresponding to 0.0021 mg/kg in matrix).</td></tr>
</table>

Conclusion

The method was found to be valid according to the guidance documents SANCO/825/00, rev 8.1 for the determination of acetamiprid in orange, sunflower seeds and maize grain with the tested LOQ of 0.01 mg/kg.

A 2.1.2.1.2 Independent laboratory validation

Comments of zRMS:	The ILV of the method has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid was successfully independently validated in oranges, sunflower seeds and maize grain. Two mass transitions were applied. Five recovery determinations were performed at two fortification levels (0,01/0,1) for each transition for each matrix. The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recoveries values in the range of 70-110% with RSD% < 10% at each fortification level for each matrix. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set in each matrix at 0,01. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Independent Laboratory Validation (ILV) of the analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices, S. P. Ticco, study no.: CH - 031/2019, 2019
Guideline(s):	Yes (SANCO/825/00, rev. 8.1 and SANCO/3029/99 rev. 4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The method described below is acceptable as ILV for the primary method "Validation of an analytical method for the determination of Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices", GLP-STUDY-18-000081; Longhi, D. (2019)". The analytical method adjusted and validated by LabAnalysis s.r.l. was shown to be specific for Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices sample.
The analysis was conducted using an HPLC hyphenated with a triple quadrupole mass detector run in multiple reaction monitoring mode. The confirmatory test has been run by following two different transitions and processing the data of both transitions, obtaining acceptable data for linearity, repeatability and recovery for each one for each matrix.

Analysis was performed under following chromatographic and mass spectrometric conditions:

Instrument	Agilent UPLC 1290 Infinity II + mass spectrometer Agilent 6470;
Column	Poroshell 120 EC - C18, 2.7 □m, 50 x 4.6 mm i.d.
Interface	Electron spray ionization (ESI), positive polarity
Detector	MS Triple quadrupole (MRM mode)
Eluent A	Water with 0.2% formic acid
Eluent B	Methanol with 0.1% formic acid
Gradient	From 95:5 A:B to 5:95 A:B in 4 minutes
Flow	0.8 mL/min;
R. T. Acetamiprid	about 3.8 minutes
Volume of injection	10 µL

Results and discussions

Table A 19 Recovery results from method validation of acetamiprid using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (*n* = 5)	Mean recovery (%)	RSD (%)	Comments
Primary transition 223/126					
Orange	Acetamiprid	0.01	105.8	2.4	
		0.1	96.2	1.53	
Sunflower seeds		0.01	95.4	3.14	
		0.1	93.7	1.73	
Maize grain		0.01	104.5	1.47	
		0.1	100.3	1.72	
Confirmatory transition 223/56					
Orange	Acetamiprid	0.01	103.9	3.77	
		0.1	97.1	2.08	
Sunflower seeds		0.01	89.1	4.34	
		0.1	94.5	1.90	
Maize grain		0.01	93.1	1.87	
		0.1	101.2	1.64	

Table A 20 Characteristics for the analytical method used for validation of acetamiprid residues in plant matrix

	Acetamiprid
Specificity	The analytical method results to be specific for Acetamiprid residues in high acid content, high oil content and dry/high starch content matrices. For each matrix, a comparison of the chromatograms of the wash (acetonitrile), the middle working standard solution (WSS 3 at 10.30 ng/mL), matrix sample and fortified matrix sample at low level (at 0.01 mg/kg) was done in order to verify possible interferences with the Acetamiprid peak. The method was demonstrated to be highly specific for the determination of Acetamiprid residues in each matrix samples by virtue of the HPLC/MS/MS technique. The analysis was conducted using the HPLC triple quadrupole in the MRM mode (two product ions at m/z 126.0 (quantifier) and 56.0 (qualifier) from the same precursor ion at m/z 223.0.

<table>
<tr><td>Calibration (type, number of data points)</td><td>For each matrix, five working standard solutions for linear calibration were prepared , using volumetric syringes, by transferring an aliquot of the second diluted standard solution into a volumetric flasks.
The linearity test was performed with working standard solutions from 1.03 ng/mL to 51.50 ng/mL concentrations of Acetamiprid for each matrix. Considering the matrices sample preparation with a nominal weight of 5.00 g, described in Appendix A, this range corresponds to an Acetamiprid residue content in each matrix samples from 0.004 mg/kg to 0.200 mg/kg.

<table><tr><td></td><td>Primary detection (MRM transition 223/126)</td><td>Confirmatory detection (MRM transition 223/56)</td></tr><tr><td>Orange</td><td>y = 748*x -507</td><td>y = 391*x – 208</td></tr><tr><td>Sunflower seeds</td><td>y = 494*x – 87</td><td>y = 259*x +10</td></tr><tr><td>Maize grain</td><td>y = 1148*x – 442</td><td>y = 594*x – 33</td></tr></table></td></tr>
<tr><td>Calibration range</td><td>Nominal range from 1.00 ng/mL to 50.00 ng/mL, corresponding to a Acetamiprid concentration ranging from 0.004 mg/kg to 0.200 mg/kg in each matrix samples.</td></tr>
<tr><td>Assessment of matrix effects is presented</td><td>yes</td></tr>
<tr><td>Limit of determination/quantification</td><td>L.O.Q. 0.01 mg/kg (or 2.575 ng/mL injected); The limit of quantification (L.O.Q.) was the low fortification level at 0.01 mg/kg, as nominal value, for Acetamiprid in each matrix samples, corresponding to a final injected solution of 2.575 ng/mL
L.O.D. 0.002 mg/kg (or 0.515 ng/mL injected); The limit of detection (L.O.D.), defined as half of the lowest calibration level, was 0.515 ng/mL, corresponding to 0.002 mg/kg for Acetamiprid in each matrix samples.</td></tr>
</table>

Conclusion

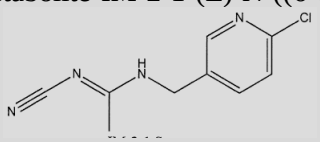
The method was found to be valid according to the guidance documents SANCO/825/00, rev 8.1 for the determination of acetamiprid in orange, sunflower seeds and maize grain with the tested LOQ of 0.01 mg/kg. The method described above is acceptable as ILV for the primary method.

A 2.1.2.1.3 Confirmatory method

No confirmatory method is required

A 2.1.2.2 Description of analytical methods for the determination of residues in animal matrices (KCP 5.2)

A 2.1.2.2.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid and metabolite IM-2-1 (E)-N-((6-chloropyridin-3-yl) methyl)- N'-cyanoacetimid-amide)  was validated in animal matrices and independently validated in milk, liver and muscle consistently with SANCO/825/00 rev.8.1 (see next study). The method is based on RAR, Miya K. (2010) and applied two transitions. The results of accuracy and precision were found in accordance with the standard validation requirements, obtaining recoveries values in the range of 70-120% with RSD% < 20% at each fortification level for each matrix. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ for acetamiprid and metabolite IM-2-1was set at 0,01. It is concluded that the analytical method in milk, liver and muscle meets the requirements of the method for post-authorization control and monitoring purposes.

Reference:	KCP 5.2
Report	Validation of the Methods of Analysis used for the determination of acetamiprid and a specified metabolite in animal commodities, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99, xxx., 2017 DNA4036,
Guideline(s):	Yes (SANCO/3029/99, rev.4)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The study objective was to validate the method of analysis used for the determination of (Acetamiprid and IM-2-1) in Eggs, Milk, Fat, Liver and Muscle in compliance with Good Laboratory Practice.

Instrument	Agilent 6470 QQQ Mass Spectrometer
Mode	Isocratic
Ionisation	Positive
Column	Phenomenex Luna-18 150 mm x 2.0 mm
Packing	Luna C-18, 3µm
Eluent	40% Acetonitrile : 60% Water with 0.1% Acetic Acid

Column Temperature	25°C
Flow Rate	0.2 ml/min
Injection Volume	1 µL
Retention Times	Approximately Acetamiprid 3.6 minutes; IM-2-1 approx. 3.0-3.1 minutes
MRM Precursor Ions	209.1 and 223.1

Results and discussions

Table A 21 Recovery results from method validation of Acetamiprid and IM-2-1 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
Liver	Acetamiprid	10	93.06	1.504	
		1.0	92.04	2.146	
		0.1	89.93	3.063	
		0.01	94.47	2.32	
	IM-2-1	10	88.55	1.401	
		1.0	86.65	2.609	
		0.1	87.16	2.453	
		0.01	87.16	1.660	
Muscle	Acetamiprid	10	91.18	1.812	
		1.0	89.57	2.801	
		0.1	90.09	1.321	
		0.01	105.2	5.565	
	IM-2-1	10	87.80	0.696	
		1.0	85.01	2.618	
		0.1	84.45	1.807	
		0.01	89.59	7.848	
Fat	Acetamiprid	10	92.24	2.182	
		1.0	90.25	2.213	
		0.1	91.07	2.034	
		0.01	96.49	2.328	
	IM-2-1	10	87.18	1.963	
		1.0	86.24	2.769	
		0.1	86.13	1.567	
		0.01	86.24	0.781	
Milk	Acetamiprid	10	93.54	1.328	
		1.0	88.78	1.030	

Matrix	Analyte	Fortification level (mg/kg) (n = x)	Mean recovery (%)	RSD (%)	Comments
		0.1	89.81	1.454	
		0.01	87.81	1.137	
	IM-2-1	10	82.38	1.521	
		1.0	80.20	1.259	
		0.1	80.31	1.542	
		0.01	80.68	1.321	
Eggs	Acetamiprid	10	99.20	0.938	
		1.0	97.26	2.420	
		0.1	98.42	1.598	
		0.01	100.4	12.28	
	IM-2-1	10	87.01	1.750	
		1.0	82.03	0.756	
		0.1	80.73	2.668	
		0.01	83.98	1.339	

Table A 22 Characteristics for the analytical method used for validation of Acetamiprid and IM-2-1 in Eggs, Milk, Fat, Liver, Muscle

	Acetamiprid and IM-2-1
Specificity	The compounds were specifically extracted from the chromatogram using accurate high resolution mass spectrometry and there were no other peaks present at the same elution time as Acetamiprid and IM-2-1
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Linear Regression Equation: Liver: y = 0.000241x – 1.876262 (Acetamiprid); y = 0.000217x -2.361351 (IM-2-1) Muscle: y = 0.000209x – 2.382621; y = 0.000195x - 2.471025 Fat: y = 0.000235x – 2.042669; y = 0.000211x – 2.421485 Milk: y = 0.000207x – 2.227308; y = 0.000193x – 2.225350 Eggs: y = 0.000277x – 0.978581; y = 0.000246x – 1.548313
Calibration range	The linearity was determined from twenty injections of ten concentrations of standard ranging from a blank to 200 µg/L Correlation coefficient Liver: 0.9980 (Acetamiprid); 0.9966

	Acetamiprid and IM-2-1
	(IM-2-1) Muscle: 0.9967 (Acetamiprid); 0.9960 (IM-2-1) Fat: 0.9982 (Acetamiprid); 0.9970 (IM-2-1) Milk: 0.9973 (Acetamiprid); 0.9970 (IM-2-1) Eggs: 0.9992 (Acetamiprid); 0.9984 (IM-2-1) These results meet the acceptance criteria of $r \geq 0.99$
Assessment of matrix effects is presented	no
Limit of determination/quantification	LOQ of 0.01 mg/kg was confirmed for Acetamiprid and IM-2-1 in animal matrices.

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 110% for mean recovery and < 20% RSD.
A highly specific detection system was used.
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4.

A 2.1.2.2.2 Independent laboratory validation

Comments of zRMS:	The ILV has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid and metabolite IM-2-1 was independently validated in milk, liver and muscle. Two transitions were applied. The results of accuracy and precision were found in accordance with the standard validation requirements, obtaining recoveries values in the range of 70-121% with RSD% < 20% at each fortification level for each matrix. Assessment of matrix effects is presented. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ for acetamiprid and metabolite IM-2-1was set at 0,01. It is concluded that the analytical method is suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Acetamiprid and its metabolite IM-2-1: Independent Laboratory Validation of an Analytical Method for the Determination in Animal Commodities, Study No. 133111101, xxx 2018
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of this study was to independently validate the analytical method "Validation of the Methods of Analysis used for the Determination of Acetamiprid and a specified metabolite in animal commodities, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99."(xxx.; 2017; Study No. DNA4036) to determine Acetamiprid and its metabolite IM-2-1 in three different matrices of animal origin, i.e. milk, liver and muscle tissue.

Instrument	Agilent Series 1290 pump and autosampler
Mass Spectrometer	API 5500
Column	Synergi 4-Hydro-RP 80A, (150 * 3 mm)
Mobile phase	60 % HPLC-H_2O + 0.1 % acetic acid 40 % acetonitrile + 0.1 % acetic acid
Detector	MSD
Ion Source	5500
Flow Rate	0.5 ml/min
Injection Volume	1 µL
Mass Transitions	Quantifier (223 m/z > 126 m/z) Qualifier (223 m/z > 90 m/z)

Results and discussions

Table A 23 Recovery results from independent laboratory validation of Acetamiprid and IM-2-1 using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
Quantifier (223 m/z > 126 m/z)					
Milk	Acetamiprid	10	120	3	
		1.0	120	2	
		0.1	114	3	
		0.01	112	4	
Qualifier (223 m/z > 90 m/z)					
Milk	Acetamiprid	10	121	3	
		1.0	121	2	
		0.1	115	3	
		0.01	120	5	
Quantifier (209 m/z > 126 m/z)					
Milk	IM-2	10	119	3	
		1.0	119	2	
		0.1	115	3	
		0.01	114	3	
Qualifier (290 m/z > 90 m/z)					
Milk	IM-2	10	119	3	

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
		1.0	119	2	
		0.1	114	3	
		0.01	117	2	
Qualifier (223 m/z > 126 m/z)					
Liver	Acetamiprid	10	112	1	
		1.0	111	1	
		0.1	103	3	
		0.01	101	6	
Qualifier (223 m/z > 90 m/z)					
Liver	Acetamiprid	10	113	1	
		1.0	112	2	
		0.1	104	4	
		0.01	104	7	
Qualifier (209 m/z > 126 m/z)					
Liver	IM-2	10	113	2	
		1.0	112	1	
		0.1	103	2	
		0.01	104	8	
Qualifier (209 m/z > 90 m/z)					
Liver	IM-2	10	112	2	
		1.0	112	2	
		0.1	101	3	
		0.01	102	6	
Qualifier (223 m/z > 126 m/z)					
Muscle	Acetamiprid	10	107	2	
		1.0	110	3	
		0.1	106	3	
		0.01	113	4	
Qualifier (223 m/z > 90 m/z)					
Muscle	Acetamiprid	10	107	2	
		1.0	109	3	
		0.1	105	3	
		0.01	104	4	
Qualifier (209 m/z > 126 m/z)					

Matrix	Analyte	Fortification level (mg/kg) (n = 6)	Mean recovery (%)	RSD (%)	Comments
Muscle	IM-2	10	107	2	
		1.0	110	3	
		0.1	106	2	
		0.01	113	2	
Qualifier (209 m/z > 90 m/z)					
Muscle	IM-2	10	106	2	
		1.0	110	4	
		0.1	105	4	
		0.01	112	4	

Table A 24 Characteristics for the analytical method used for independent laboratory validation of Acetamiprid and IM-2-residues in animal matrices

	Acetamiprid and IM-2-1
Specificity	There was no interference from blank values and therefore the recommendation by SANCO guideline (< 30 % of the mean peak area at LOQ level) is fulfilled.
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. The LC-MS/MS responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99), for more details and every calibration graph please refer to the study report.
Calibration range	1.2 to 200 µg/L – Milk 1.2 to 40 µg/L (low range) 1.2 to 200 µg/L (high range) – Liver; Muscle The calibration was performed using calibration solutions. These results meet the acceptance criteria of r ≥ 0.99.
Assessment of matrix effects is presented	~~No~~ yes
Limit of determination/quantification	**Acetamiprid** Limit of Detection (LOD): Quantifier: 0.092 µg/L corresponding to 0.230 µg/kg milk Qualifier: 0.689 µg/L corresponding to 1.722 µg/kg milk Limit of Quantification (LOQ): 0.01 mg/kg milk **IM-2-1** (LOD): Quantifier: 0.504 µg/L corresponding to 1.260 µg/kg milk Qualifier: 0.157 µg/L corresponding to 0.391 µg/kg milk (LOQ): 0.01 mg/kg milk **Acetamiprid** (LOD): Quantifier: 0.080 µg/L corresponding to 0.200 µg/kg Qualifier: 0.655 µg/L corresponding to 1.636 µg/kg (LOQ): 0.01 mg/kg liver **IM-2-1** (LOD): Quantifier: 0.391 µg/L corresponding to 0.978 µg/kg liver Qualifier: 0.144 µg/L corresponding to 0.360 µg/kg liver (LOQ): 0.01 mg/kg liver **Acetamiprid** (LOD): Quantifier: 0.206 µg/L corresponding to 0.514 µg/kg meat Qualifier: 0.533 µg/L corresponding to 1.333 µg/kg meat

	Acetamiprid and IM-2-1
	(LOQ): 0.01 mg/kg meat **IM-2-1** (LOD): Quantifier: 0.366 µg/L corresponding to 0.915 µg/kg meat Qualifier: 0.376 µg/L corresponding to 0.939 µg/kg meat (LOQ): 0.01 mg/kg meat

Conclusion

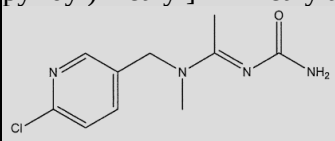
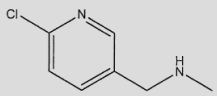
In conclusion, the analytical method for the determination of acetamiprid and IM-2-1 in milk, liver and in muscle tissue was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfill the demanded validity criteria. For the method validation purpose, the matrices milk, liver and muscle tissue were spiked at four concentration levels and an LOQ of 0.01 mg/kg was established for each matrix.

A 2.1.2.2.3 Confirmatory method

No confirmatory method is required

A 2.1.2.3 Description of Methods for the Analysis of Soil (KCP 5.2)

A 2.1.2.3.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid and IM-1-4, IM-1-2 metabolites was successfully validated in soil. The IM-1-2 metabolite is also known as (E)-N^2-carbamoyl-N^1-[(6-chloro-3-pyridyl) methyl]-N^1-methylacetamidine and has a structure:  The IM-1-4 metabolite is also known as 1-(6-Chloro-3-pyridinyl)-N-methyl-methanamine and has a structure:  Transitions for quantitation and confirmation were applied. It was found for IM-1-4 metabolite that 157→128 transition provided no response and was probably an error in the methodology. However, the 157→73.1 was a strong response and this was considered finally a more realistic transition in the method as the confirmation ion. Calcareous soil supplied by LGC was used as matrix to obtain all the recovery parameters and show that the methodologies were specific to Acetamiprid, IM-1-4 and IM-1-2, with no background matrix inferences from the specific matrix type. Five recovery determinations were performed at two fortification levels for each matrix. The results of accuracy and precision were found in accordance with the requirements in the range of 70-110% with RSD% < 20% at each fortification level (except 1 case). The independent validation (see next study) confirms the validity of this method. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0.002 mg/kg for each analyte. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1., D.Norris, Study code: DNA4517, 2018
Guideline(s):	SANCO/825/00, rev. 8.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The objective of the study was to validate the method of analysis used for the determination of the test items Acetamiprid and two Acetamiprid Metabolites (IM-1-4 and IM-1-2) in soil according to the guideline SANCO/825/00 rev. 8.1. The analysis were performed by LC-QQQ.

Instrumental Parameters:

HPLC	Agilent 6470 QQQ Mass Spectrometer
Analytical column	Phenomenex Aqua-C18, 150mm x 2.0 mm
Flow	0.5 ml/min
Injection volume	10 µL
Column temperature	25 °C
Eluent	60% Acetonitrile : 40% Water with 1.0% Acetic Acid
Ionization	positive

Results and discussions

Table A 25 Recovery results from method validation of Acetamiprid and two Acetamiprid Metabolites (IM-1-4 and IM-1-2) using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
Soil	Acetamiprid	0.02	92.62	4.14	
		0.002	86.52	29.62	
Soil	IM-1-4	0.02	81.69	1.98	
		0.002	85.23	11.52	
Soil	IM-1-2	0.02	89.57	2.18	
		0.002	94.32	6.31	

Table A 26 Characteristics for the analytical method used for validation of Acetamiprid, IM-1-4 and IM-1-2

	Acetamiprid	IM-1-4	IM-1-2
Specificity	Acetamiprid eluted at 4.3 minutes. The compound was speciffically extracted from the chromatogram using MRM mass spectrometry, and there were no other peaks present at the same elution time as Acteamipryd.	IM-1-4 eluted at 3.3 minutes. The compound was speciffically extracted from the chromatogram using accurate high resolution mass spectrometry, and there were no other peaks present at the same elution time as IM-1-4	IM-1-2 eluted at 2.5 minutes. The compound was speciffically extracted from the chromatogram using MRM mass spectrometry, and there were no other peaks present at the same elution time as IM-1-2.
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Calibration curve: y= 0.000407*x-0.111513	Linear calibration function was obtained with correlation coefficient > 0.99. Calibration curve: y= 0.000074x-0.059334	Linear calibration function was obtained with correlation coefficient > 0.99. Calibration curve: y= 0.000729*x+680364*x-0.035113

	Addidional calibration functions: y= 0.000464*x-0.020211		
Calibration range	The linearity was determined from sixteen injections of eight concentrations of standards. Correlation coefficient: r= 0.996733 Additional correlation coefficient r= 0.999856 These results meet the acceptance criteria of r ≥ 0.99	Correlation coefficient: r= 0.999617 These results meet the acceptance criteria of r ≥ 0.99	Coefficient of determination: r^2= 0.999602 These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	No matrix effect		
Limit of determination/quantification	LOQ=0.002 mg/kg (Acetamiprid, IM-1-4, IM-1-2)		

Conclusion

Results of the recovery experiments indicate that the recovery efficiency and repeatability were within acceptable limits of 70% - 110% for mean recovery and < 20% RSD.
A highly specific detection system was used.
The analytical method therefore meets the requirements of guideline SANCO/3029/99 rev. 4 and SANCO/825/00, rev. 8.1.

A 2.1.2.3.2 Independent laboratory validation

Comments of zRMS:	The ILV of the method has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid and its metabolites IM-1-2 and IM-1-4 was successfully independently validated in soil. Two mass transitions were applied. Six recovery determinations were performed at two fortification levels (0,002/0,02) for each transition for each matrix. The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recoveries values in the range of 70-110% with RSD% < 20% at each fortification level for each matrix. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0,002. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Acetamiprid and its Metabolites IM-1-2 and IM-1-4: Independent Laboratory

	Validation of an Analytical Method for the Determination in Calcareous Soil. 133113101, Eichler M., Herrmann S., 2018
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of this study was to independently validate the analytical method "Validation of the Methods of Analysis used for the Determination of Acetamiprid and two Acetamiprid Metabolites in Calcareous Soil, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1."(Norris, D.; 2018; Study No. DNA4517) to determine Acetamiprid and its metabolites IM-1-2 and IM-1-4 in calcareous soil. The targeted LOQ for this method was 0.002 mg/kg soil.
The analytes were extracted from soil by means of three different solvent mixtures. The filtered extracts were combined, made up to volume and a subsample taken for LC-MS/MS analysis.
The method was validated with respect to linearity, specificity, accuracy and precision. For method validation blank samples as well as fortified samples were analysed.

Instrument	Agilent Series 1290 pump and autosampler
Mass Spectrometer	API 5500
Column	Synergi 4-Hydro-RP 80A, (150 * 3 mm)
Mobile phase	60 % HPLC-H_2O + 1 % acetic acid 40 % acetonitrile + 0.1 % acetic acid
Detector	MSD
Ion Source	5500
Flow Rate	0.5 ml/min
Injection Volume	5 µL
Mass Transitions	Quantifier (223 m/z > 126 m/z) Qualifier (223 m/z > 90 m/z)

Results and discussions

Table A 27 Recovery results from method validation of Acetamiprid and two Acetamiprid Metabolites (IM-1-4 and IM-1-2) using the analytical method

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
quantifier (223 m/z > 126 m/z)					
Soil	Acetamiprid	0.02	90	1	
		0.002	86	4	
quantifier (223 m/z > 90 m/z)					
Soil	Acetamiprid	0.02	89	2	
		0.002	95	10	

Matrix	Analyte	Fortification level (mg/kg) (n = 5)	Mean recovery (%)	RSD (%)	Comments
quantifier (241 m/z > 126 m/z)					
Soil	IM-1-2	0.02	103	3	
		0.002	85	3	
quantifier (241 m/z > 198 m/z)					
Soil	IM-1-2	0.02	104	3	
		0.002	87	5	
quantifier (157 m/z > 126 m/z)					
Soil	IM-1-4	0.02	90	6	
		0.002	88	6	
quantifier (157 m/z > 73 m/z)					
Soil	IM-1-4	0.02	n.d	n.d	
		0.002	n.d	n.d	

Table A 28 Characteristics for the analytical method used for validation of Acetamiprid, IM-1-4 and IM-1-2

	Acetamiprid	IM-1-4	IM-1-2
Specificity	Specificity was established by monitoring two different mass transitions for acetamiprid: • quantifier 223 → 126 m/z • qualifier 223 → 90 m/ There was no interference from blank values.	Specificity was established by monitoring two different mass transitions for acetamiprid: • quantifier 157 → 126 m/z • qualifier 157 → 73 m/z There was no interference from blank values.	Specificity was established by monitoring two different mass transitions for IM-1-2: • quantifier 241 → 126 m/z • qualifier 241 → 98 m/z There was no interference from blank values.
Calibration (type, number of data points)	Linear calibration function was obtained with correlation coefficient > 0.99. Calibration Curves: Quantifier: y = 69198 * x + 103 Qualifier: y = 19364 * x - 42	Linear calibration function was obtained with correlation coefficient > 0.99. Calibration curve: Quantifier: y = 209990* x + 328 Qualifier: not applicable	Linear calibration function was obtained with correlation coefficient > 0.99. Quantifier: y = 128725 * x + 2884 Qualifier: y = 89167 * x + 3047
Calibration range	Calibration Range: 0.03 to 5 µg/L Linearity of Response: Quantifier: 0.9999 Qualifier: 1.0000 These results meet the acceptance criteria of r ≥ 0.99	Calibration Range: 0.03 to 2 µg/L Linearity of Response: Quantifier: 1.0000 Qualifier: not applicable These results meet the acceptance criteria of r ≥ 0.99	Calibration Range: 0.03 to 10 µg/L Linearity of Response: Quantifier: 0.9999 Qualifier: 0.9999 These results meet the acceptance criteria of r ≥ 0.99
Assessment of matrix effects is presented	Matrix effects were eliminated by preparation of matrix-matched standards as was done in original method DNA4517.		
Limit of	LOQ=0.002 mg/kg (Acetamiprid, IM-1-4, IM-1-2)		

determination/quantification	

Conclusion

In conclusion, the analytical method for the determination of acetamiprid, IM-1-4, IM-1-2 in calcareous soil was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfil the demanded validity criteria.

A 2.1.2.3.3 Confirmatory method

No confirmatory method is required.

A 2.1.2.4 Description of Methods for the Analysis of Water (KCP 5.2)

A 2.1.2.4.1 Method validation

Comments of zRMS:	The validation has been accepted. In this study the method for determination of acetamiprid in drinking water was validated (also independently validated – see next studies). The LC-MS/MS method was taken from RAR 07, Miya K. (2010). The acetamiprid molecular ion used was 223m/z and the fragment ions used were 56m/z, 99m/z and 126m/z. The linearity was determined from eighteen injections of nine concentrations of standard ranging from a blank to 100 ug/L acetamiprid. The recovery samples were prepared for analysis at 10 µg/L, 1.0 µg/L, 0.1 µg/L and 0.05 µg/L. All obtained validation parameters were included within the required range consistently with SANCO/825/00 rev. 8.1. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0.05 µg/L. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Validation of the Methods of Analysis used for the determination of acetamiprid in water, in Compliance with Good Laboratory Practice, Norris, D, Study Number: DNA4037, 2017
Guideline(s):	Yes (SANCO/3029/99)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The validation parameters for the Acetamiprid in Water methodology have been met for this study under the SANCO/3029/99 guidelines.. Each analyte was directly determined by Liquid Chromatography (LC-QQQ).

Instrument	Agilent 6470 QQQ Mass Spectrometer
Mode	Isocratic
Column	YMC-Pack ODS-AQ, (150 * 3 mm)
Mobile phase	60 % Water with 0.1% Acetic Acid 40 % Acetonitrile
Packaging	5 µm
Column temperature	25°C
Flow Rate	0.5 ml/min
Injection Volume	2 µL
Gas Temperature	230°C

Results and discussions

Table A 29 Recovery results from method validation of Acetamiprid using the analytical method

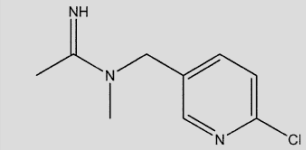
Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	Acetamiprid	10	92.20	1.537	
		1.0	93.09	3.602	
		0.1	91.79	1.423	

Table A 30 Characteristics for the analytical method used for validation of Acetamiprid residues in drinking water

	Acetamiprid
Specificity	Acetamiprid eluted at 2.9 minutes. The compound was speciffically extracted from the chromatogram using accurate high resolution mass spectrometry, and there were no other peaks present at the same elution time as Acetamiprid.
Calibration (type, number of data points)	The LC-QQQ responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99)
	Calibration curve: y= 0.002521* x – 0.752574
Calibration range	The calibration was performed using calibration solutions (9 concentrations) within the range from blank to 100 µg/L.
	The linear correlation coefficients (r) for this data set were 0.999433.
Assessment of matrix effects is presented	No matrix effects
Limit of determination/quantification	The limits of quantification (LOQs) of the analytical method were confirmed at 0.05 µg/L in drinking water for Acetamiprid.

Conclusion

The validation parameters for the Acetamiprid methodology have been met for this study under the SANCO/3029/99 guidelines.

Comments of zRMS:	The validation has been accepted. In this study the method for determination of IM-1-5 metabolite (N-(6-chloro-pyridin-3-ylmethyl)-N-methylacetamidine)  in drinking water was validated (also independently validated – see next studies). The method was taken from Giesau, A and Weber, H (2012). The analysis was conducted by direct injection of drinking water samples. The mixture was fortified and analysed on the LC-MS/MS. The method provided a good peak shape using isocratic elution – this was not considered a significant deviation from the original method. The following mass transitions were monitored: IM-1-5 m/z 198.1 — 126.0 (quantification), m/z 198.1 — 99.0 (confirmation). The sum of both ion responses was used for quantification. The water used to conduct all the spiked recovery parameters showed that the methodologies were specific to metabolite IM-1-5 with no background matrix inferences from the specific matrix type. The linearity was determined from twenty injections of ten concentrations of standard ranging from a blank to 5.0 µg/L of metabolite IM-1-5. The recovery samples were prepared for analysis at 0.5 µg/L and 0.05 µg/L. All obtained validation parameters were included within the required range. LOQ was set at 0.05 µg/L. There were no peaks present in the two drinking water extracts or the solvent blank at the same elution time as IM-1-5. This demonstrates that there were no analyte interferences and the method is specific to IM-1-5.

Reference:	KCP 5.2
Report	Validation of the Methods of Analysis used for the Determination of a Metabolite of Acetamiprid in Drinking Water, Norris D., Study Number: DNA4518, 2018
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The validation parameters for the Acetamiprid in Water methodology have been met for this study under the SANCO/3029/99 guidelines. Each analyte was directly determined by Liquid Chromatography (LC-QQQ).

Instrument	Agilent 6470 QQQ Mass Spectrometer
Mode	Isocratic
Column	Phenomenex, (150 * 3 mm)

Mobile phase	80 % Water with 1.0% Acetic Acid 20 % Acetonitrile
Packaging	Aqua 5 µm
Column temperature	25°C
Flow Rate	1.0 ml/min
Injection Volume	20 µL
Gas Temperature	230°C

Results and discussions

Table A 31 Recovery results from method validation of IM-1-5

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Drinking water	IM-1-5	0.5	107.3	1.07	
		0.05	113.4	1.47	

Table A 32 Characteristics for the analytical method used for validation of IM-1-5

	IM-1-5
Specificity	IM-1-5 eluted at 1.4 minutes. The compound was specifically extracted from the chromatogram using MRM mass spectometry, and there were no other peaks present at thj same rlution time as IM-1-5.
Calibration (type, number of data points)	The LC-QQQ responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99) Calibration curve: y= 0.0000067* x + 0.0103442
Calibration range	The calibration was performed using calibration solutions (10 concentrations) within the range from blank to 5 µg/L. The linear correlation coefficients (r) for this data set were 0.9973312.
Assessment of matrix effects is presented	No background matrix inferences from the specific matrix type.
Limit of determination/quantification	LOQ=0.05 µg/L

Conclusion

In conclusion, the analytical method for the determination of IM-1-5 have been met for this study under the SANCO/825/00 rev. 8.1 guidelines.

A 2.1.2.4.2 Independent laboratory validation

Comments of zRMS:	The ILV of the method has been accepted.

	In this study the analytical LC-MS/MS method for determination of acetamiprid was successfully independently validated in drinking water. Two mass transitions were applied. There was no interference from blank values. The peak areas obtained from measurement of the solvent standards and matrix-matched standards were compared. The mean difference between peak areas was 7 % for quantifier and qualifier mass transition. Six recovery determinations were performed at 4 fortification levels (0.05 µg/L /0.1 µg/L/1 µg/L/10 µg/L) for each transition for each matrix. The results of accuracy and precision were found in accordance with the SANCO/825/00 rev.8.1 requirements, obtaining recoveries values in the range of 70-110% with RSD% < 20% at each fortification level for each matrix. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0.05 µg/L. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Acetamiprid: Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water, M. Eichler, Herrmann, S., Study No. 133112101, 2018
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of this study was to independently validate the analytical method "Validation of the Methods of Analysis used for the Determination of Acetamiprid in Water, in Compliance with Good Laboratory Practice, and referencing SANCO/3029/99."(Norris, D.; 2017; Study No. DNA4037) to determine Acetamiprid in drinking water.
The analyte was extracted from drinking water using a Mega Bond Elut C18 and a Sep-Pak plus C18 solid phase extraction (SPE) cartridge. The eluate was evaporated to dryness under reduced pressure and reconstituted in solvent. The analyte concentration in the final solutions was determined via LC-MS/MS technique.

Instrument	Agilent Series 1290 pump and autosampler
Mass Spectrometer	API 5500
Column	Synergi 4-Hydro-RP 80A, (150 * 3 mm)
Mobile phase	60 % HPLC-H2O + 1 % acetic acid 40 % acetonitrile + 1 % acetic acid
Flow Rate	0.5 ml/min
Injection Volume	5 µL
Detector	MSD

Results and discussions

Table A 33 Recovery results from method validation of Acetamiprid

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
223 m/z > 126 m/z; for quantitation					
Drinking water	Acetamiprid	0.05	82	3	
		0.1	84	6	
		1	91	8	
		10	89	1	
223 m/z > 90 m/z; for confirmation					
Drinking water	Acetamiprid	0.05	73	3	
		0.1	80	5	
		1	92	8	
		10	91	1	

Table A 34 Characteristics for the analytical method used for validation of Acetamiprid residues in drinking water

	Acetamiprid
Specificity	Specificity was established by monitoring two different mass fragments, one as a quantifier (223 → 126 m/z) and one as a qualifier (223 → 90 m/z).
Calibration (type, number of data points)	The LC-QQQ responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99)
	Calibration curve: Quantifier: y = 121513 * x + 35366; Qualifier: y = 32570 * x + 15768
Calibration range	Calibration range: 0.7 to 100 µg/L Aliquots of the stock solution were diluted with acetonitrile / pure water (50/50, v/v) to obtain eight intermediate solutions.
	The linear correlation coefficients (r) for this data set were 0.9999.
Assessment of matrix effects is presented	The peak areas obtained from measurement of the solvent standards and matrix-matched standards were compared. The mean difference between peak areas was 7 % for quantifier and qualifier mass transition. Therefore, it was decided to use calibration standards prepared from solvent for calibration and evaluation of samples.
Limit of determination/quantification	The limits of quantification (LOQs) of the analytical method were confirmed at 0.05 µg/L in drinking water for Acetamiprid.

Conclusion

In conclusion, the analytical method for the determination of the test item in drinking water was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfill the demanded validity criteria.

Independent laboratory validation

Comments of zRMS:	The ILV of the method has been accepted. In this study the analytical LC-MS/MS method for determination of metabolite IM-1-5 was successfully independently validated in drinking water. Two mass transitions were applied. Six recovery determinations were performed at two fortification levels (0.05 µg/L /0.5 µg/L) for each transition. The results of accuracy and precision were found in accordance with the requirements, obtaining recoveries values in the range of 70-110% with RSD% < 20% at each fortification level. The method was validated regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0.05 µg/L. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	IM-1-5 (Metabolite of Acetamiprid): Independent Laboratory Validation of an Analytical Method for the Determination in Drinking Water, Study No. 133141101, 2018
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The purpose of this study was to independently validate the analytical method "Validation of the Methods of Analysis used for the Determination of a Metabolite of Acetamiprid in Drinking Water, in Compliance with Good Laboratory Practice, and referencing SANCO/825/00 rev. 8.1."(Norris, D.; 2018; Study No. DNA4518) to determine IM-1-5 (Metabolite of Acetamiprid) in drinking water.
The targeted LOQ for this method was 0.05 µg/L drinking water.
The analysis of the metabolite IM-1-5 in drinking water was conducted by direct injection of the spiked and control samples. The analyte concentration in the final solutions was determined via LC-MS/MS technique.

Instrument	Agilent Series 1290 pump and autosampler
Mass Spectrometer	API 5500
Column	Acquity UPLC BEH C18 (100*2.1 mm; 1.7 µm)
Mobile phase	80 % HPLC-H_2O + 1 % acetic acid 20 % acetonitrile + 1 % acetic acid
Flow Rate	0.45 ml/min
Injection Volume	20 µL

Detector	MSD

Table A 35 Recovery results from method validation of IM-1-5

Matrix	Analyte	Fortification level (µg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments	
quantifier m/z 198 amu → 126 amu						
Drinking water	IM-1-5	0.05	98	6		
		0.5	101	5		
qualifier m/z 198 amu → 90 amu						**qualifier m/z 198 amu → 90 amu)**
Drinking water	IM-1-5	0.05	99	6		
		0.1	103	1		

Table A 36 Characteristics for the analytical method used for validation of IM-1-5 residues in drinking water

	IM-1-5
Specificity	Specificity was established by monitoring two different mass fragments, one as a quantifier (223 → 126 m/z) and one as a qualifier (223 → 90 m/z).
Calibration (type, number of data points)	The LC-QQQ responses were shown to be linear throughout the entire study (correlation coefficients (r) > 0.99)
	Calibration curve: Quantifier: y = 1801305*x+ 91989; (198 → 126 m/z; quantifier) – high range Qualifier: y = 501952*x+ 17203; (198 → 90 m/z; qualifier) – high range y = 2188640 * x- 2478 - Calibration Curve (198 → 126 m/z; quantifier) – low range y = 559202 * x+ 130 - Calibration Curve (198 → 90 m/z; qualifier) – low range
Calibration range	Calibration ranges: 0.015 to 5 µg/L (high range) 0.015 to 0.25 µg/L (low range)
	Quantifier (high): 0.9991; Qualifier (high): 0.9995; Quantifier (low): 0.9997; Qualifier (low): 0.9999
Assessment of matrix effects is presented	The peak areas obtained from measurement of the solvent standards and matrix-matched standards were compared. The mean difference between peak areas was 30%. Therefore, it was decided to use the matrix-matched calibration standards for calibration and evaluation.
Limit of determination/quantification	The limits of quantification (LOQs) of the analytical method were confirmed at 0.05 µg/L in drinking water for IM-1-5.

Conclusion

In conclusion, the analytical method for the determination of the test item in drinking water was independently validated in this project. Results for specificity, linearity, accuracy and precision are given and fulfill the demanded validity criteria.

A 2.1.2.4.3 Confirmatory method

No confirmatory methods are required.

A 2.1.2.5 Description of Methods for the Analysis of Air (KCP 5.2)

A 2.1.2.5.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical method for determination of acetamiprid was successfully validated in air. After sonication, an aliquot of the organic solvent was analysed in a LC-MS/MS with 2 transitions: m/z 223 to m/z 126 (primary quantifier) and m/z 223 to m/z 56 (secondary confirmation). The method was validated consistently with SANCO/825/00 rev.8.1 guideline regarding recovery, repeatability, LOQ, specificity and linearity. The LOQ was set at 0.002 µg/m^3. The matrix effect was proved to be less than 20%. It is concluded that the analytical method was shown to be suitable for the assigned purposes.
Reference:	KCP 5.2
Report	Validation of an analytical method for the determination of Acetamiprid residues in air, D. Longhi, Study No. GLP-STUDY-18-000080, 2019
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of this Study was the Validation of an analytical method for the determination of Acetamiprid in air using glass fibre filters as sorbent material.
The validated method consisted in a simple extraction of the analyte from the filters with acetonitrile. After sonication, an aliquot of the organic solvent was filtered and injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and m/z 223 to m/z 56 (secondary confirmation detection 223/56).

Instrument	Agilent UPLC 1290 Infinity II
Mass Spectrometer	Agilent 6470
Column	Zorbax Eclipse Plus C18, 1.8 µm × 2.1 mm × 50 mm

Mobile phase	A: 0.1 % v/v formic acid in Q1 water (prepared dissolving a volume of 1 mL of formic acid in 1 L of Q1 water); Mobile phase B: 0.1 % v/v formic acid in methanol (prepared dissolving a volume of 1 mL of formic acid in 1 L of methanol for UPLC/MS);
Flow Rate	0.4 mL/min;
Injection Volume	2 µL
Column temperature	40°C

Table A 37 Recovery results from method validation of Acetamiprid

Matrix	Analyte	Fortification level (µg/m³) (n = x)	Mean recovery (%)	RSD (%)	Comments
Extraction efficiency test results – Primary transition 223/126					
Air	Acetamiprid	0.002	103.7	1.4	
		0.02	87.6	1.6	
Extraction efficiency test results – Confirmatory transition 223/56					
Air	Acetamiprid	0.002	103.1	0.8	
		0.02	87.2	1.9	
Overall recovery test results – Primary transition 223/126					
Air	Acetamiprid	0.002	93.2	2.2	
		0.02	83.4	3.3	
Overall recovery test results – Confirmatory transition 223/56					**qualifier m/z 198 amu → 90 amu)**
Air	Acetamiprid	0.002	94.3	2.5	
		0.02	83.6	2.4	

Table A 38 Characteristics for the analytical method used for validation of Acetamiprid residues in air

	Acetamiprid
Specificity	The method was found to be selective for the determination of the analyte Acetamiprid for both the monitored transitions, that gave very similar results. No interfering signals were detected in the untreated matrix in amount higher than 30% of the LOQ level, that complies with the guideline requirements.
Calibration (type, number of data points)	Calibration curve: Primary detection: y = 2182.58*x – 63.92 R^2=0.9996 Confirmatory detection: y = 1056.3*x – 18.18

	Acetamiprid
	R^2=0.9997
Calibration range	The linearity was evaluated on the calibration range from 0.624 ng to 41.6 ng (that are equal to the range from 0.00065 to 0.043 µg/m3 of air considering a fluxing volume of 0.96 m3) in 6 levels, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56.
Assessment of matrix effects is presented	~~No~~ Yes. In order to compensate for possible matrix effects, calibration was generated using standards prepared in blank matrix extracts (matrix-matched standards).
Limit of determination/quantification	The limit of quantification of this method is 0.0020 µg/m3 of Acetamiprid

Conclusion

The applied analytical method was validated under GLP compliance according to the SANCO/825/00 rev.8.1 guideline.

A 2.1.2.6 Description of Methods for the Analysis of Body Fluids and Tissues (KCP 5.2)

A 2.1.2.6.1 Method validation

Comments of zRMS:	The method has been accepted. In this study the analytical LC-MS/MS method for determination of acetamiprid residues was validated in blood. Two transitions were applied. The method was validated consistently with SANCO/825/00 rev.8.1 guideline regarding recovery, repeatability, LOQ, specificity and linearity. The linearity was evaluated in 5 levels, monitoring both transitions. The linearity was checked analysing matrix matched standard solutions. The LOQ was set at 0.05 mg/L. It is concluded that the analytical method was shown to be suitable for the assigned purposes.

Reference:	KCP 5.2
Report	Validation of an analytical method for the determination of Acetamiprid residues in blood, xxx, Study No. GLP-STUDY-18-000079, 2019
Guideline(s):	Yes (SANCO/825/00 rev. 8.1)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Materials and methods

The aim of this Study was the Validation of an analytical method for the determination of Acetamiprid in

bovine blood. The validated method consisted in a 2-times-extraction of the analyte from the matrix with acetone. After centrifugation, an aliquot of the organic supernatant was filtered, dried under a nitrogen stream, dissolved in acetonitrile/water, filtered and injected in a UPLC-MS/MS system for the final determination, setting the instrument in the multi reaction monitoring mode (MRM) on 2 transitions: m/z 223 to m/z 126 (primary quantifier detection 223/126) and m/z 223 to m/z 56 (secondary confirmation detection 223/56).

Instrument	Agilent UPLC 1290 Infinity II
Mass Spectrometer	Agilent 6470
Column	Zorbax Eclipse Plus C18, 1.8 µm × 2.1 mm × 50 mm
Mobile phase	A: 0.1 % v/v formic acid in Q1 water (prepared dissolving a volume of 1 mL of formic acid in 1 L of Q1 water); Mobile phase B: 0.1 % v/v formic acid in methanol (prepared dissolving a volume of 1 mL of formic acid in 1 L of methanol for UPLC/MS);
Flow Rate	0.4 mL/min;
Injection Volume	2 µL
Column temperature	40°C

Table A 39 Recovery results from method validation of Acetamiprid

Matrix	Analyte	Fortification level (mg/L) (n = x)	Mean recovery (%)	RSD (%)	Comments
Recovery Test Results – Primary transition 223/126					
Whole bovine blood	Acetamiprid	0.002	100.9	2.0	
Recovery Test Results – Confirmatory transition 223/56					
Whole bovine blood	Acetamiprid	0.002	101.6	3.2	

Table A 40 Characteristics for the analytical method used for validation of Acetamiprid residues in blood

	Acetamiprid
Specificity	This parameter was evaluated in order to demonstrate that the applied method detects the right analyte and that the analytical signal is quantitatively correct not affected by other compounds. Using a MS/MS mass spectrometer detector, the selectivity was evaluated comparing the following chromatograms: a blank, a sample, a fortified sample and a reference solution at the LOQ level, in order to assess the presence or absence of interfering signals. No interfering signals were detected in the untreated matrix in amount higher than 30% of the LOQ level.
Calibration (type, number of data points)	Calibration curve: Primary detection: y = 1633.67*x – 160.42 R^2=0.9999 Confirmatory detection: y = 788.70*x – 81.25 R^2=0.9997

	Acetamiprid
Calibration range	The linearity was evaluated on the same calibration range from 2.082 to 20.82 µg/L (equal to from 0.0260 to 260 mg/L in the blood samples) in 5 levels, monitoring both the MRM transitions: 223/126 and the confirmatory 223/56.
Assessment of matrix effects is presented	~~No~~ Yes. In order to compensate for possible matrix effects, calibration was generated using standards prepared in blank matrix extracts (matrix-matched standards).
Limit of determination/quantification	The limit of quantification of this method is 0.05 mg/L of Acetamiprid

Conclusion

The applied analytical method was validated under GLP compliance according to the SANCO/825/00 rev.8.1 guideline.

A 2.1.2.7 A.2.A.9 Other Studies/ Information

No new or additional studies have been submitted